ACIDOSIS AND GROWTH

DOUGLAS GREGG BOYDEN

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A C I D O S I S A N D G R O W T H

by

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Dedicated To

Dr. MABEL G. BOYDEN

and

Dr. ALAN A. BOYDEN

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The study of growth is a subject which has fascinated workers in medicine and its allied sciences for many years. Numerous experimental techniques have been devised for altering the rate of growth of animals and for studying the effect of various nutritional factors on animals whose growth has been so altered. This research has been extremely fruitful and has contributed much to our modern concept of nutrition.

These experiments have one main factor in common. An altered rate of growth is produced by a deficiency of certain dietary factors; when this deficiency is replaced, the normal rate of growth is resumed. The clinical applications of the results of these experiments is obvious. The causes of such diseases as scurvy, rickets, pellagra, and beri-beri are now understood and rational therapy may be instituted.

Factors other than dietary ones are known to have marked influences on growth. It is commonly observed that children with congenital heart disease are smaller in stature than normal children of the same age. This dwarfism is not pronounced, but its existence is unquestioned and its etiology is unknown (Landtman, 1947).

Hormonal factors are of paramount importance in maintaining proper growth. The anterior pituitary gland occupies a central position in the regulation of growth, mediated both by the pituitary growth hormone (PGH) and by the various tropic factors effecting the thyroid, adrenals and gonads.

Lack of PGH in childhood causes dwarfism of the Loraine-Levi type, while increased production of this hormone by the anterior pituitary causes gigantism. Lack of thyroid hormone during the growth period of humans causes cretinism, characterized by stunting, infantilism, and retarded mental development. Hyperfunction of the adrenals causes dwarfism, perhaps due to the effects of the adrenal steroids on protein metabolism.

Increased amounts of androgens, as for example occur in the hormone therapy of cryptorchism, or in Leydig cell tumors, cause premature closure of epiphyses, and thus result in short stature. Moreover, removal of the testes in childhood, as in eunuchoidism, results in abonormally tall stature.

Lucas first called attention to the occurrence of rickets in children with renal disease. Writing in the Lancet, 1883, he states:

> "The phenomena of late rickets and albuminuria are too frequently connected to be matters of chance, and the latter is often an important symptom indicating the cause of the former."

The literature is singularly devoid of further reference to this interesting clinical entity until 1911, at which time Parsons and Fletcher each published one case. Barber (1920) clearly defined the syndrome of dwarfism and rickets associated with renal disease and introduced the term "renal dwarfism" for this symptom complex.

Miller and Parsons published a series of cases of renal dwarfism in 1912. These workers considered dysfunction of the hypophysis to be the main cause of this disease.

Green (1922) first commented upon the occurrence of acidosis in renal dwarfism, although he, along with Shipley, Park, et al (1922) considered the acidosis to be due to dietary causes, i.e. starvation acidosis.

Lathrop, in 1926, described one case of renal dwarfism and considered that phosphate retention was the cause of the faulty bone growth seen in this disease.

In 1927, Parsons divided renal dwarfism into three main types on the basis of roentgen findings. These were 1) Florid, characterized by X-Ray findings similar to those observed in florid rickets; 2) Atrophic, characterized by generalized osteoporosis; and 3) Wooly, characterized by a "moth-eaten" appearance of the bone.

Ellis and Evans, in 1933, called attention to the occurrence in renal dwarfism of dilation in the urinary tract, and proposed a theory involving some dysfunction of the autonomous nervous system; i.e. achalasia of the urethro-vesical sphincter as the etiology of the renal insufficiency.

Chown, in 1936, elaborated this theory further on the basis of one case, and considered a dysfunction of the hypothalamus to be the chief cause of the disorder.

Shelling and Remsen, in 1936, considered the chief cause of renal rickets to be the decreased ability of the diseased kidney to excrete phosphorus. This phosphorus is then shifted to the gut for excretion, and there combines with ingested calcium, thus decreasing absorbtion of calcium. They also consider acidosis to play an important part in the causation of this disease.

Follis, in 1943, published a series of thirty-nine cases of chronic renal disease in adults, in which half showed skeletal changes typical of osteomalacia in the vertebral bodies.

Prior to 1950, there was a difference of opinion as to whether the skeletal changes in renal dwarfism were true rickets. The latest work (Follis, 1950) seems to indicate that true rickets, characterized by faulty calcification of osteoid tissue, actually does occur in renal dwarfism.

Renal dwarfism is a syndrome characterized by retarded physical and mental development, polydipsia, polyuria, skeletal abnormalities, and anemia, occurring in individuals who suffer from chronic renal insufficiency. This renal disease may either be congenital or acquired. In the former category are conditions such as polycystic kidneys, or hereditary metabolic disorders involving the renal tubules, such as DeToni-Fanconi syndrome, and cysteinosis. In the latter category, are renal diseases such as interstitial fibrosis secondary to pyelonephritis, chronic glomerulonephritis, or obstructive uropathy due to strictures, adhesions, or masses in the urinary tract. The skeletal changes are those of rickets in children, and osteomalacia in adults. Serum studies show increased levels of non-protein nitrogen, inorganic phosphorus, and lipid, and decreased calcium, carbon dioxide content, and pH.

There have been many theories proposed concerning the etiology of renal dwarfism. Some have stood the test of time well; others have been consigned to limbo by newer discoveries. The field may be described as fluid at present.

The most widely held theories regarding renal dwarfism may be classified into three main groups: 1) Nervous, 2) Endocrine, 3) Metabolic.

1) Nervous

Ellis and Evans, in 1933, published twenty cases of renal dwarfism. In seventeen of these, post mortem examinations were performed and fourteen of these showed certain dilatations of the urinary tract. These authors then advanced the theory that renal dwarfism was caused by an imbalance of the autonomic nerves which supplied the lower urinary tract, causing a functional obstruction thereof.

Chown (1936) described two cases of renal dwarfism in one of which serial sections showed a hemi-agenesis of the hypophysis. He agreed with Ellis and Evans that imbalance of the autonomic system might be a contributing factor in renal dwarfism, but he placed the basic pathology in the hypothalamus. According to Chown, a basic pituitary-hypothalamic dysfunction causes delayed bone calcification. This delay so deranges calcium and phosphorus metabolism that these ions tend to precipitate out in the kidney, thus causing chronic interstitial nephritis. This theory rests on the assumption that the pituitary regulates bone calcification. Chown admits that this regulation would be hard to prove experimentally because of the plethora of factors which influence skeletal calcification. Incidentally, it is interesting to note that the main subjective symptoms of polydipsia, polyuria, and dwarfism can be produced by pituitary malfunction.

2) Endocrine

The fact that parathyroid hyperplasia occurs in renal disease has long been known. Mac Callum, in 1905, first noted this hyperplasia and his work was soon confirmed, both clinically and experimentally, by others.

Parathyroid hyperplasia in renal disease may be either primary or secondary. In the former, there is usually a benign solitary adenoma, and the blood reflects the effect of increased parathormone by showing low phosphorus and high calcium concentrations. As a result of the increased serum calcium, this ion tends to precipitate out in certain tissues, notably the kidney, thus causing interstitial nephritis. Parathyroid hyperplasia secondary to renal disease, however, is usually found to involve all four glands and thought by some to be caused by a rise in serum phosphate concentration due to renal retention of this ion. Along with increased serum phosphate, there is decreased serum calcium. Skeletal changes in both types of parathyroid hyperplasia are those of osteitis fibrosa.

3) Metabolic

The interest of workers investigating renal dwarfism has been directed to calcium and phosphorus metabolism because of the prominence of skeletal changes in this disease. As a result of such work, two theories of the cause of renal rickets have been evolved, both having as basis the inability of the diseased kidney to excrete phosphate.

Mitchell and his associates (1930-33) proposed the following theory to account for the retarded growth and skeletal changes observed in renal dwarfism. There is a primary renal insufficiency which may either be congenital or acquired. Such diseased kidneys can no longer excrete sufficient phosphorus to maintain normal serum concentration of this ion. Therefore, the excretion of phosphorus is re-routed from the kidneys to the gut and the excess phosphorus is lost into the lumen of the gastro-intestinal tract.

There it combines with ingested calcium, to form insoluble calcium phosphate, which effectively reduces absorbtion of calcium and causes actual calcium starvation.

Other workers have discovered evidence supporting this idea. Diets with marked relative increase of calcium (with normal phosphorus) or of phosphorus (with normal calcium) are rachitogenic in animals. That is, excess ingestion of either calcium or phosphorus interferes with absorbtion of the other ion (Mitchell and Guest, 1933).

Boyd, Courtney, and MacLachlan (1926) observed a reciprocal relationship between urinary and fecal phosphorus, and as phosphorus in feces increased, there was a proportional increase in fecal calcium.

Ford (1931) found parallel increases in fecal calcium and phosphorus of nephritic children.

The acidosis which almost invariably accompanies renal dwarfism has also received its share of attention. The pH of the extra cellular fluid is regulated within fairly narrow limits by both respiratory and renal mechanisms.

The respiratory mechanism acts through its effect on the carbonic acid-bicarbonate buffer system. Carbon dioxide, produced in body metabolism, is dissolved in body fluids with the aid of the enzyme carbonic anhydrase (CA) according to the following equation:

$$CO_2 + H_2O \xleftrightarrow{CA} H_2CO_3$$

Whenever carbon dioxide tension in vascular fluids is increased, the respiratory center is stimulated and the excess carbon dioxide is lost through the lungs. The mechanism of the stimulus is unknown.

The fixed cations of the body fluids are sodium and potassium. The fixed anions of the body fluids are mainly chloride, with inorganic phosphate and sulphate being less important. These ions can be neither synthesized nor destroyed in the body. Since the concentration of these ions cannot change rapidly enough to meet the varying needs of the body under differing metabolic conditions, acute regulation of the pH of body fluids is taken care of by the volatile ions, especially carbonic acid. Thus, increase in fixed cation (sodium) relative to fixed anion (chloride) calls for increase of volatile anion (carbonic acid) and vice versa.

The renal mechanisms are concerned with the selective reabsorbtion of ions from the glomerular filtrate. In simplified terms, the regulation of pH of extracellular fluid depends on the relative reabsorbtion of sodium and chloride by the kidney tubules. Increased reabsorbtion of sodium over chloride conserves fixed cation and is the main defense against acidosis.

The kidney has two ways of conserving fixed base:

1. The titrable acidity of the urine is increased.
    a. Hydrogen ions are substituted for the sodium in such salts as sodium acetate and sodium citrate.
    b. Dibasic sodium phosphate is changed to the monobasic salt by substitution of hydrogen ion for one of the sodium ions.
2. Ammonia is manufactured from glutamine in the kidney and is substituted for sodium in the tubular fluid.

The hydrogen ions of the first two equations come from the dissociation of carbonic acid and the bicarbonate thus supplied is reabsorbed with sodium. That this mechanism of substituting hydrogen ions for sodium is important is shown by studies involving an inhibitor of the enzyme

carbonic anhydrase. This substance, acetazoleamide (Diamox) has been shown to decrease the substitution of hydrogen ions for sodium, thus causing losses of fixed cation and water. In fact, its clinical use as a diuretic depends on this reaction (Leaf, Relman, and Schwartz, 1954).

In acidosis, all these mechanisms come into play. If the acidosis is of such a degree that these mechanisms are not sufficient to bring extra-cellular pH to normal, fixed cation is lost in order to excrete increasing amounts of acid. These fixed cations are chiefly sodium, with potassium and calcium of less importance. The minerals of bone provide a substantial reservoir of fixed cations. Large amounts of sodium and smaller amounts of calcium have been shown to be mobilized from bone in acidosis (Bergstrom and Wallace, 1954).

In the diseased kidney, the ability to conserve fixed cation by substituting hydrogen and ammonium ions for sodium is decreased, and there is a tendency toward phosphorus retention and acidosis. The high inorganic phosphorus, low calcium and pH of the serum produce a medium in which there is reduced calcification of osteoid in children, and decalcification of bone in adults.

Ginzler and Jaffe (1941) and Albright (1937) consider the acidosis to be the most important factor of the production of skeletal changes and retarded growth in renal dwarfism. Graham and Oakley (1938) treated two cases of renal rickets with large amounts of base along with Vitamin D and observed striking improvement in the rachitic bones of their patients. Vitamin D alone has no effect in renal rickets. Hamilton and Dewar (1937) observed that sodium salts of citric and tartaric acid prevented rickets in rats on rachitogenic diets. They attributed this action to the ingestion of fixed cation, i.e. sodium.

Because of the central position of acidosis in renal dwarfism, it was considered worth while to investigate the role of acidosis per se on growth.

Group	No. Pairs of Animals	Experimental Procedure	Duration of Experiment, Days 1st Period	2nd Period
IA	7	Unilateral nephrectomy and 4% NH_4Cl	32	15
IB	6	Unilateral nephrectomy	32	18
II	11	4% NH_4Cl and 0.2% Diamox	40	14
III	12	13% CO_2	38	16
IV	10	0.4% Diamox	42	

Figure I. Plan of Experiment.

METHODS

Thirty-day old albino male rats of the Sprague Dawley strain were used. These were maintained in individual cages 7" by 7" by 9", of one-half inch wire mesh. Water and purina fox chow were allowed ad libitum for a short period of eight to ten days to determine the normal rate of growth and to insure that all the animals were healthy. The environmental temperature was maintained at 25°C with only slight variation.

After this preliminary period, the rats were arbitrarily separated into four groups and each animal was paired with the one nearest to it in weight within a given group. At this time, the animals' ears were notched to give a positive means of identification.

The feed in all cases was ground fox chow, to which the necessary amount of the materials under study was added. This feed was given to the rats in small round tins 3" by 1½" in size with closed covers, which were wired to the floor of the cage. Access to the feed was provided by a hole 1½" in size, surrounded by a large flange, which admitted only the rats' head.

All animals received water ad libitum. Experimental animals received feed ad libitum. This feed was weighed daily and a like amount of feed was given to the control animals. Thus, the control animals received, in one day, the amount of feed consumed during the previous day by the experimental animals. Allowances were made for wastage. No allowance was made for 4 per cent of the diet which, in experimental animals, was electrolyte (NH_4Cl) in Groups IA and II. It was considered that this amount of feed was not significant, especially as the feed was weighed only to one gram.

All animals were weighed every other day on a torsion balance scale accurate to 0.1 gm.

At intervals during the early experimental period, samples of tail blood were collected under oil for chloride analysis to determine the level of acidosis. The concentration of acid-producing substances in the diet was modified upward until a satisfactory acidosis was produced and this concentration was maintained until the end of the experimental period.

All animals showing signs of upper respiratory infection were excluded from the experiment.

Experimental Groups

IA. Six Experimental Animals and Six Controls
Unilateral Nephrectomy and 4% NH_4Cl

At the start of this experiment, the experimental animals were anesthetized with ether. The left kidney was then removed through a left lumbar paravertebral incision. There was little hemorrhage and the animals withstood the procedure very well. These rats were then placed on a diet containing 4 per cent ammonium chloride. At the end of the experiment, four experimental animals and two control animals were sacrificed for serum, muscle and bone analysis, and the survivors were placed on normal diets.

IB Seven Experimental Animals and Seven Controls
Unilateral Nephrectomy

The experimental animals in this group underwent the same operative procedure as those in Group IA. However, they were maintained on normal diet. Four experimental and two control animals were sacrificed at the end of the experiment, and the remainder were kept.

II Eleven Experimental Animals and Eleven Controls
4% NH_4Cl and 0.1% Diamox

These animals were started on a diet containing 1 per cent ammonium chloride. This diet did not produce a satisfactory acidosis, as shown by serum chloride determinations. Therefore, the amount of ammonium chloride was increased to 2 per cent, and 0.05 per cent Diamox was added. This diet also produced an unsatisfactory acidosis, and consequently the amount of both these substances was doubled. The final diet contained 4 per cent ammonium chloride and 0.1 per cent Diamox.

At the termination of the experiment, seven experimental animals and three controls were sacrificed for analysis. The survivors were placed on a diet calculated to be neutral, which contained 4 per cent ammonium chloride, 0.1 per cent Diamox, 2 per cent potassium bicarbonate, and 7 per cent sodium bicarbonate.

III Twelve Experimental Animals and Twelve Controls
13% CO_2

The experimental animals in this group were maintained in a clear lucite box, approximately 2' by 2' by 2'. One side was removable for access to the individual cages in which the rats were kept. This side was sealed by a rubber gasket. The seal was broken once each day for weighing feed and animals and for cleaning the cage. Excess moisture in the chamber was absorbed by a beaker of concentrated sulphuric acid. Compressed carbon dioxide and compressed room air were led into a small mixing chamber by rubber tubing and thence into the center of the chamber. This atmosphere was analyzed once each day for carbon dioxide. Average value of carbon dioxide content in the air was 13 per cent (Henderson Haldane). Oxygen determinations were also made. Average value of oxygen was 17 per cent (Roughton Scholander).

At the termination of this experiment, seven experimental animals and three controls were selected for analysis. These animals were anesthetized and their heads were placed in a cone of stiff paper, to which the gas mixture was led by rubber tubing. This device served to maintain the serum pCO_2 while the animals were being exsanguinated. The survivors were then maintained in room air.

IV Ten Experimental Animals and Ten Controls
0.4% Diamox

The experimental animals in this group were placed on a diet of 0.2 per cent Diamox. Two pairs of animals were sacrificed for serum determinations of sodium, potassium, carbon dioxide, and chloride. These indicated only a slight degree of acidosis, so the amount of Diamox in the diet was doubled and maintained at 0.4 per cent. After forty-two days, all animals were sacrificed.

In order to rule out defects of absorbtion of protein or fat as a cause of retarded growth, a group of three rats was made acidotic with 4 per cent ammonium chloride and 0.1 per cent Diamox. Feed uptake was weighed and all feces over a three-day period were collected, dried for forty-eight hours at 105°C, weighed, pulverized, and analyzed for nitrogen and fat. A control group of three normal rats was similarly treated.

Because rats are nocturnal animals, they consume most of their feed at night. In view of this fact, it was considered advisable to analyze the serum for pH and electrolytes at different hours of the day to determine if the rats who were made acidotic by ingestion of ammonium chloride or Diamox could escape from the influence of these substances during the daylight hours. This was important because the animals in Groups I to IV were sacrificed from 9:00 a.m. to 3:00 p.m.

Therefore, eight rats were made acidotic with 0.4 per cent Diamox and two animals were sacrificed at approximately four hour intervals from 10:00 a.m. to 11:00 p.m. and serum was analyzed for pH, sodium, potassium, carbon dioxide and chloride.

At the termination of the above experiments, selected animals were anesthetized by intra-peritoneal injection of 5 mg nembutal per 100 grams of rat, in physiological saline. The animals were then exsanguinated anaerobically by puncturing the abdominal aorta with hypodermic needle and syringe. The calf and leg muscles were removed for analysis, care being taken to include as little fat as possible. The long bones (humerus, femus, and tibia) were removed and freed of periosteum and epiphyses. The following determinations were performed:

Serum: pH, sodium, potassium, chloride, carbon dioxide content, and phosphorus.

Bone: Sodium, potassium, chloride, and calcium.

Muscle: Sodium, chloride, potassium, inorganic phosphorus, and fat.

These analyses were performed by accepted methods in use in the Laboratory of Pediatrics of Yale University School of Medicine.

The length of the femur, exclusive of epiphyses, was measured to 0.1 mm with a pair of vernier calipers.

The kidneys, adrenals, and thyroid were meticulously dissected free, weighed to 0.1 mg on an analytical balance, and preserved in Zenkers solution for histologic study. The distal end of the femur was likewise preserved for sectioning.

One pair of animals from each of the first four groups of animals was X-rayed.

The surviving animals were then placed back under normal conditions to determine if the changes experimentally produced were reversible.

Calculations

Weight gain in grams, feed uptake in grams, and nutritional efficiency (weight gain divided by feed uptake) were calculated for every animal, both for the initial experimental period and the final normal period.

The weights of kidneys, adrenals and thyroid were expressed in absolute values (gm or mg) and relative values (gm or mg per 100 gram rat).

Electrolytes of serum were expressed in mEq/L, save for phosphorus, which was expressed in mg/%. Electrolytes of muscle were expressed as mM per 100 grams of fat free solid. Electrolytes of bone were expressed as mEq per 1000 gm of solid.

The sodium and potassium of bone were corrected for the Donnan effect on charged particles and the amount of solids in serum. In addition, these electrolytes were corrected for the amount of sodium and potassium in the vascular fluids in bone.

Because potassium is mostly intracellular, the Donnan effect and corrections for the amount of proteins in the serum were assumed to be negligible.

All chloride was assumed to be extracellular. The chloride concentration per liter of interstitial fluid is determined by equation (1):

$$(1) \qquad [\text{Interstitial Chloride}] = \frac{[\text{Chloride}]_{\text{serum}}}{940 \times .95} \times 1000$$

Then the extracellular water is determined by equation (2):

$$(2) \quad \text{Extracellular } H_2O = \frac{\text{Total bone chloride}}{\text{Interstitial Chloride}}$$

The amount of sodium per liter of interstitial fluid is calculated by equation (3):

$$(3) \quad \frac{[\text{Serum Na}] \times .95}{940} \times 1000 = \text{Interstitial Na}$$

Corrections were then made for sodium and potassium to eliminate that fraction of these ions in the vascular fluids by equations (4) and (5):

$$(4) \quad \text{Extracellular Na} = \text{Extracellular } H_2O \times [\text{Interstitial Na}]$$

$$(5) \quad \text{True bone Na} = \text{Total bone Na} - \text{Extracellular Na.}$$

All data enumerated above were averaged separately for each group of experimental and control animals. The standard deviation and standard error of the mean were then calculated. Utilizing these figures, the standard error of the difference between experimental mean and control mean was then calculated. If this difference was greater than three times its standard error, it was considered statistically significant.

RESULTS

General Observations

One animal in Group I and three in Group II showed signs of upper respiratory infection manifested by wheezing, sniffling, and lethargy. These animals were etherized to prevent spread of the illness and they were excluded from the results.

The other animals were maintained well on their various acid-producing regimens. The experimental animals in Group III suffered anorexia while in 13 per cent carbon dioxide and did not eat normal amounts of feed. This decreased feed uptake caused the control animals in this group to be ravenously hungry and to gain less weight than the controls in the other groups.

There was no observable difference in activity between the experimental and control animals in Groups I, II, and IV, nor were the experimental animals in these groups obviously hyperpneic. In Group III, the animals, while in 13 per cent carbon dioxide, usually lay curled up in their cages and were obviously hyperventilating. As soon as the cages were removed from carbon dioxide for cleaning, however, the animals first cleaned themselves off thoroughly and then scampered about in their cages, apparently emjoying the fresh air. Their controls showed activity comparable to that of the animals in Groups I and II.

Group IA — Unilateral Nephrectomy and 4% NH_4Cl
Data for this group appear in Figures 2,3,6, and 7

Growth

The experimental animals in Group IA gained an average of 3.0 grams per day during the initial experimental period of thirty-two days for an average total weight gain of 96.2 grams. During this time, they consumed an average of 21.6 grams of feed daily, or 693 grams of feed for the entire experimental period. The ratio of weight gain per unit of feed consumed, hereafter called nutritional efficiency, was 0.141.

The control animals, while consuming the same amount of feed, gained an average of 5.2 grams per day, for a total average weight gain of 166.4 grams, and a nutritional efficiency of 0.202. Thus, the control animals gained 1.7 times as much weight as the experimental animals on the same feed intake.

A glance at the growth curves discloses the pattern of growth for both experimental and control animals. It will be noted that both groups of animals gained at the same rate until the experimental animals underwent nephrectomy. At that time, these animals suffered a slight lag in growth, but soon recovered and at eight days post-operative, they were gaining at the same rate as their controls. At ten days post-operative, however, 4 per cent ammonium chloride was added to their diet and their growth immediately slowed down, so that they gained 60 per cent of their total weight increment in the ten days from nephrectomy until institution of 4 per cent ammonium chloride in diet, and only 40 per cent in the twenty-two days from then until the termination of the experiment.

During the second part of the experiment, the only change was the removal of ammonium chloride from the diet. During this period of fifteen days, the experimental animals consumed an average of 24.7 grams of feed daily for a total of 372 grams. They gained an average of 2.1 grams per day for an average total weight gain of 31.2 grams. The nutritional efficiency was 0.083. The controls, meanwhile, although they consumed the same amount of feed, gained an average of approximately 1 gram daily for a total weight increment of 14.7 grams. The nutritional efficiency was 0.043.

The growth curves demonstrate a spurt in growth for the experimental animals with a gradual leveling off of the slope of the curve, so that at the end of the period, both experimental and control animals were gaining at the same rate. The apparent loss of weight of the control animals on the median curves is due to a statistical artifact caused by shifting the median from six animals to two animals.

Organs

The average absolute weight of the kidney of the experimental animals equaled 1.7280 grams, while the two kidneys of the controls averaged 2.7160 grams in weight. Thus, in the experimental animals, the remaining kidney had hypertrophied to approximately 64 per cent of the normal renal mass. The relative weight of the kidney (per 100 gram rat) in the experimental animal averaged 0.668 grams, while in the control animals, the relative weight of both kidneys averaged 0.814 grams. Thus, when the weight of the kidney is related to total body weight, the experimental animals had, in one kidney, 82 per cent of the renal mass of the normal animals with two kidneys.

The absolute weight of the adrenals in the experimental animals averaged 62.3 mg, while the adrenals of the controls averaged 48.8 mg in weight. The relative weight of the adrenals (per 100 gram rat) averaged 24.1 mg in the experimental animals, and 16.3 mg in the controls. Thus, the adrenals were 43 per cent heavier in the experimental animals than in the controls.

The average weight of the thyroid in the experimental animals was 18.5 mg, that of the controls was 22.3 mg. No significant difference was found between the relative weight (per 100 gram rat) of the thyroid glands of experimental animals (7.0 mg) and that of controls (6.4 mg).

Femur Length

Average femur length, exclusive of epiphyses, of the experimental animals, was 29.1 mm; that of controls averaged 30.8 mm. Thus, the femurs of the controls were 5.8 per cent longer than those of the experimental animals.

Histology

No differences were observed between sections of control and experimental thyroid glands. Follicles were lined with low columnar epithelium and were filled with normal colloid.

Sections of thyroid glands of three experimental and one control animal in Group IA revealed parathyroid tissue. No differences were noted in the histological structure of this gland between control and experimental animals.

The adrenal glands of both experimental and control animals revealed normal histologic structure as described by Nicander (1954).

No marked differences were observed in the distal end of the femur between experimental and control animals. Cortices and trabeculae were normal. There was no thickening of the epiphysial line in the experimental animals. There were no signs of abnormal osteoclastic activity.

The kidney of the experimental animals showed diffuse hypertrophy, characterized by increased size of the organ, slight dilatation of the secretory tubules, and increase in size and vascularity of the glomeruli.

Roentgen Examination

Radiologic examination of one pair of animals showed only minimal changes between experimental and control animals. These changes were limited to a slight decrease in radio-density of the skeleton of the experimental animals and minimal coarsening of the trabeculae, the latter best demonstrated at the proximal ends of humeri and tibiae. No noticeable difference was observed in the widths of the epiphyseal cartilage between experimental and control animals.

Electrolytes

Serum:

Serum electrolyte determinations revealed a slight hyperchloremic acidosis in the experimental animals; pH values for the experimental animals averaged 7.42, while those of the controls averaged 7.46, certainly no significant difference.

The carbon dioxide content of the serum in the experimental animals averaged 22.7 mEq/L and the chloride concentration of the serum averaged

103.1 mEq/L. The serum carbon dioxide content of the controls, on the other hand, averaged 24.9 mEq/L, and the chloride concentration of the serum averaged 96.5 mEq/L.

No significant differences were observed for sodium, potassium, and inorganic phosphorus between experimental and controls animals.

Muscle:

Analyses of muscle revealed that the sodium concentration of the acidotic rats was below that of the controls. Thus, the average muscle sodium of acidotic animals was 7.76 mM per 100 gram fat free solid, while that of the controls was 8.07 mM per 100 gram fat free solid. No significant difference was noted between the average content of muscle potassium in the acidotic animals (45.9 mM per 100 gram fat free solid) and in the controls (45.2 mM per 100 gram fat free solid). Likewise, the average concentration of muscle inorganic phosphorus and chloride were almost identical in the experimental animals and in the controls.

There was slightly less fat in the muscle of acidotic animals than in the controls. Thus, the average weight of fat was 5.12 grams per 100 grams solid in the acidotic animals, and 5.09 grams per 100 grams solid in the controls.

Bone:

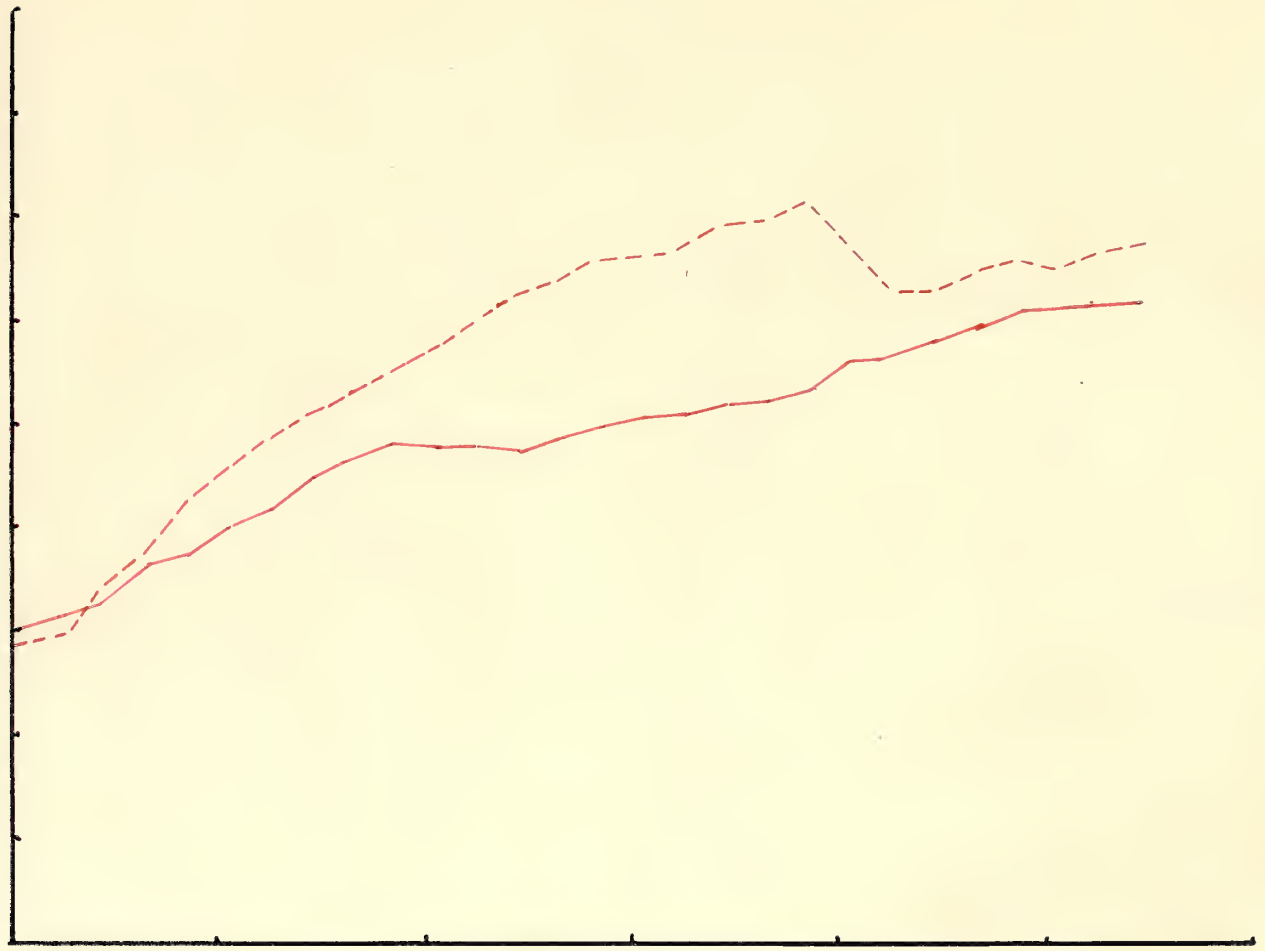
Analyses of bone electrolytes revealed that the calcium content of bone in the experimental animals was lower than that of the controls. Thus, bone calcium averaged 7204 mEq/Kg of fresh bone in acidotic animals, vs. 7481 mEq/Kg fresh bone in the controls.

Bone sodium in acidotic animals (126 mEq/Kg fresh bone) averaged somewhat lower than in control animals (133 mEq/Kg fresh bone). However, there was no significant difference between corrected bone sodium values for acidotic and control animals. There was no significant difference in average bone potassium content between acidotic animals (44.2 mEq/Kg fresh bone) or controls (47.0 mEq/Kg fresh bone). This also held true for corrected bone potassium.

Bone chloride averaged lower in the acidotic animals (14.3 mEq/Kg fresh bone) than in controls (23.9 mEq/Kg fresh bone).

There was considerable variation from the mean in individual determinations of bone electrolytes, especially with regard to sodium.

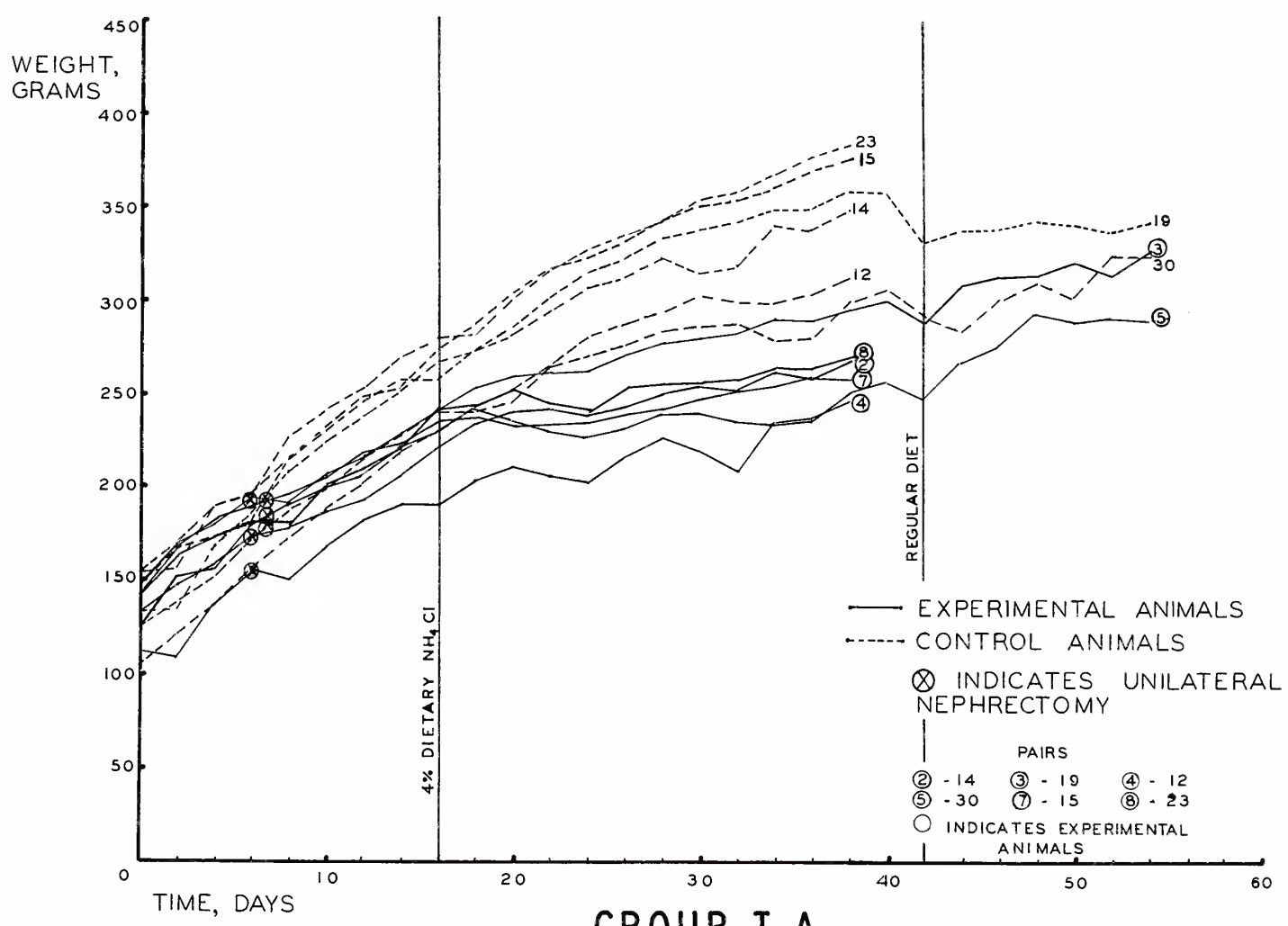
Figure 2. Individual growth curves of all animals.
The red curves show median growth for experimental and control animals.



MEDIAN WEIGHT VS TIME

MEDIAN WEIGHT VS TIME

GROWTH DATA



GROUP I A

UNILATERAL NEPHRECTOMY
+ 4% DIETARY NH_4Cl

Figure 3. Radiogram of animals [illegible]. Experimental animal on right, control on left. Note decreased density of tibiae on experimental animal and coarsening of trabeculae in proximal tibial epiphyses.

Group IB Unilateral Nephrectomy
Data for this group appear in Figures 4 to 7 inclusive

Growth

The experimental animals in this group gained an average of 4.6 grams per day for the initial experimental period of thirty-two days, making a total average weight gain of 140.6 grams. During this period, they consumed an average of 21.6 grams of feed daily for a total average feed uptake of 690 grams. Average nutritional efficiency was 0.205.

The control animals, restricted to the same feed uptake, gained an average of 4.8 grams daily, for a total average weight gain of 150.1 grams. Their nutritional efficiency averaged 0.222. Thus, there was a difference in weight gain of only 7 per cent between experimental and control animals in this group.

During the second period of eighteen days, the surviving animals were maintained on the same regimen, inasmuch as no dietary acid-producing factors were utilized in this group. The experimental animals gained an average of 2.1 grams daily, for a total average increment of 37.8 grams. They consumed an average of 25.8 grams of feed daily, or 466 grams for the entire period. Their average nutritional efficiency was 0.081.

Meanwhile, the controls, on the same amount of feed, gained an average of 0.9 grams daily, or 16.9 grams for the second period. The nutritional efficiency of the controls averaged 0.036.

The growth curves disclose several points of interest with regard to the pattern of growth. It will be noted that both groups of animals grew at approximately the same rate until the experimental animals

underwent surgery. At that time, these animals suffered a setback and did not gain weight at the same rate as their controls until twelve days post-operative. Then, although still lighter, they gained weight at a faster rate than their controls, and gradually caught up with them. At the end of the first experimental period, both groups of animals weighed approximately the same and were growing at the same rate.

During the second period, both experimental and control animals grew at approximately the same rate as during the initial period. The apparent loss of weight of the control animals in the median curves is mostly statistical artifact, as it will be noted from the individual curves that two of the three animals lost no weight.

Organs

The kidneys of the experimental animals averaged 1.7090 grams in weight. The two kidneys of the controls weighed an average of 2.6977 grams. Thus, the experimental animals had 64 per cent of the renal mass of the controls. The weight of kidney per 100 grams body weight averaged 0.560 gm in the experimental animals and 0.783 in the controls. Thus, the experimental animals had 71 per cent of the renal mass of their normal controls per unit of body weight.

The weight of adrenals in the experimental animals averaged 62.8 mg, while the adrenals of the controls averaged 74.6 mg in weight. The weight of adrenal tissue per 100 gm body weight averaged 20.5 mg in the experimental animals and 21.4 mg in the controls. Thus, no significant difference in the weight of adrenals per unit of body weight was noted between experimental and control animals.

Thyroid weight averaged 28.0 mg for the experimental animals and 22.6 mg for the controls. The experimental animals had 9.2 mg thyroid tissue per 100 gm body weight, while the controls had 6.5 mg thyroid per 100 gm body weight.

No significant difference was observed in the length of femur between experimental and control animals. Thus, femur length in the experimental animals averaged 30.0 mm, while the femurs of control animals averaged 30.5 mm length.

Histology

Sections of thyroid gland of both experimental and control animals showed normal structure, with follicles lined with low columnar epithelium and filled with normal colloid.

One section of thyroid, in an experimental animal, revealed normal parathyroid tissue.

Sections of adrenal glands of both experimental animals and controls revealed normal histologic structure.

Sections of the distal end of the femur showed no evidences of osteoporosis, osteitis fibrosa, or rickets in the experimental animals. Both groups of animals showed normal cortices, trabeculae, and epiphysial plates. No evidences of increased osteoclastic activity were observed.

Sections of kidney in all experimental animals showed evidences of hypertrophy, characterized by increased size of the kidney, slight dilatation of secretory tubules, and increase in size of glomeruli. These changes, however, did not seem so marked as in Group IA.

Roentgen Examination

Radiological examination of one pair of animals revealed no changes whatsoever between experimental animals and controls.

Electrolytes

Serum:

No marked differences in the electrolyte structure of the serum were observed between experimental and control animals.

Thus, Serum pH averaged 7.33 in both groups of animals. Sodium, potassium, and inorganic phosphorus showed no consistent differences between experimental and control animals. There was a slight decrease in carbon dioxide content in the experimental animals. Carbon dioxide content in these animals averaged 27.3 mEq/L, while that of the controls averaged 29.4 mEq/L. There was a difference in chloride concentration of only 1 mEq/L between the two groups of animals. Thus, chloride concentration in the experimental animals averaged 96.9 mEq/L, while that of the controls averaged 95.9 mEq/L.

Muscle:

Analyses of muscle electrolytes revealed a slightly higher sodium (average 8.77 mM per 100 gram fat free solid) in the experimental animals than in the controls (average 8.53 mM per 100 gram fat free solid). No significant differences were noted between experimental and control animals in concentrations of muscle potassium, phosphorus, and chloride. There was a marked difference in fat content of the muscle between experimental and control animals. Thus, experimental animals averaged 7.34

gram fat per 100 gram solid, while the controls averaged 3.83 gram per 100 gram solid. However, this latter value is undoubtedly due to error of some kind, as no other control animals had values so low. It is probably of no significance.

Bone:

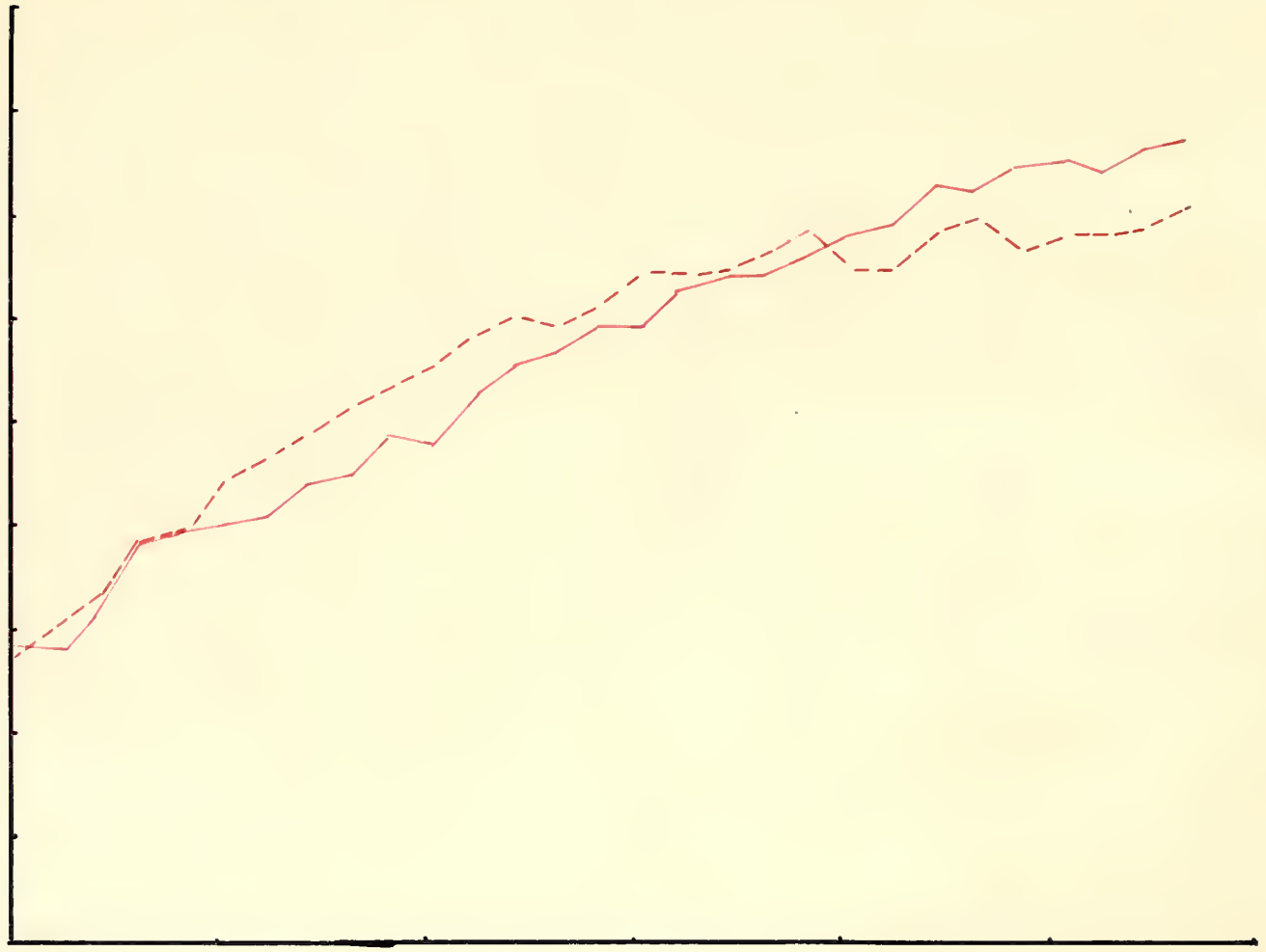
Average bone calcium was slightly higher in the experimental animals (7761 mEq/Kg fresh bone) than in the controls (7623 mEq/Kg fresh bone).

Average bone sodium was lower in the experimental animals than in the controls. Thus, the experimental animals had an average of 125 (corrected 99) mEq sodium per Kg fresh bone, while the controls had an average of 160 (corrected 139) mEq sodium per Kg fresh bone. The same held true for potassium. The experimental animals had an average of 34.2 (corrected 28.1) mEq of potassium per Kg fresh bone, whereas the controls averaged 43.4 (corrected 37.4) mEq potassium per Kg fresh bone.

Average chloride content, however, was higher in the experimental animals (19.5 mEq/Kg fresh bone) than in the controls (15.5 mEq/Kg fresh bone).

There was, however, considerable variation from the average in the individual determinations of bone electrolytes.

Figure 4. Individual growth curves of all animals. The red curves show median growth for experimental and control animals.

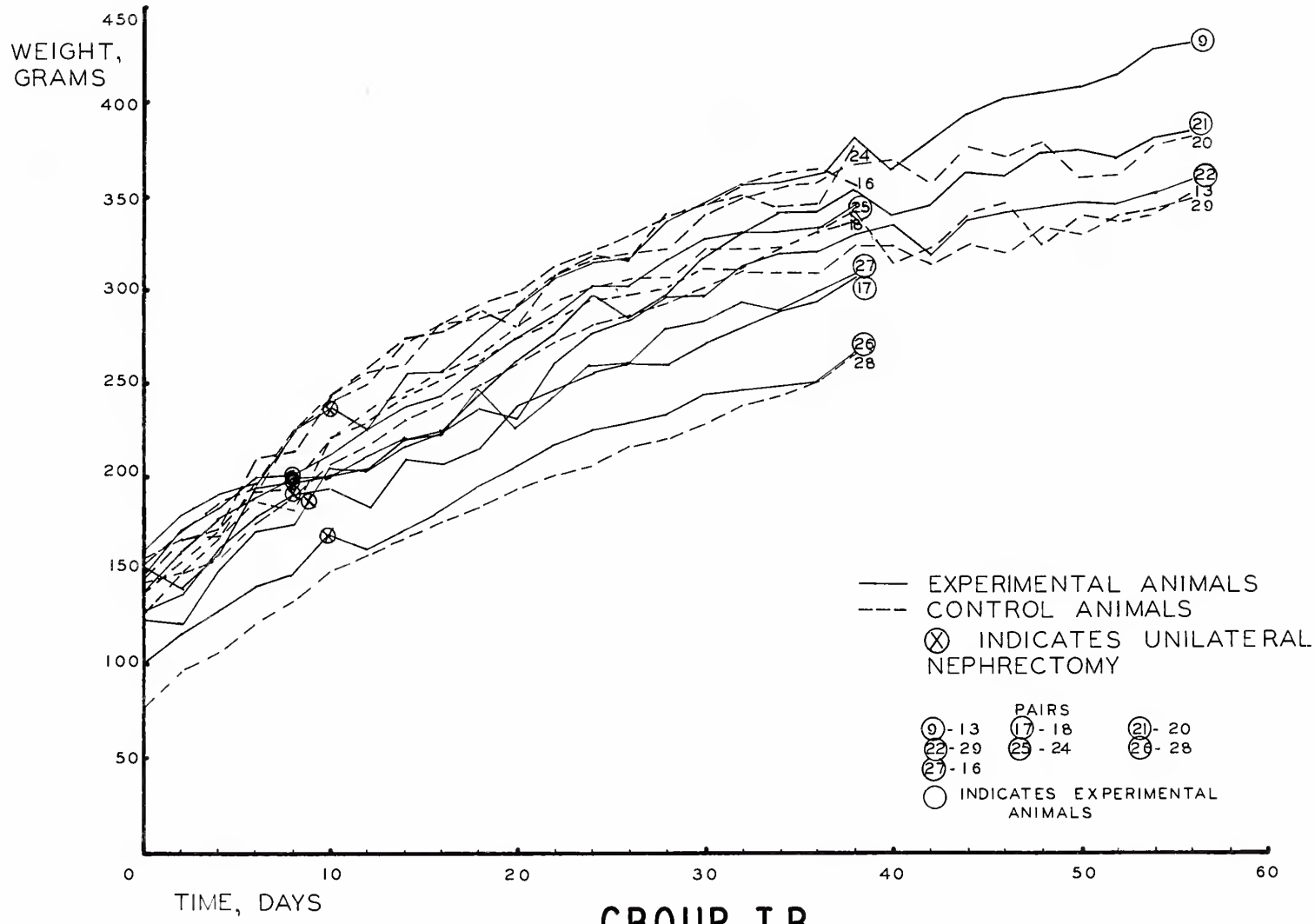


MEDIAN WEIGHT VS TIME

MEDIAN WEIGHT VS TIME

GROWTH DATA

WEIGHT vs TIME



GROUP IB

UNILATERAL NEPHRECTOMY

Figure 5. Radiogram of animals 21 and 20. Experimental animal on right, control on left. No marked differences in skeletal density are noted in these two animals.

Figure 6. Growth, feed uptake, and organ data.

SUMMARY OF DATA

RAT NUMBER	WEIGHT GAIN, GM.	FEED UPTAKE, GM.	GRAMS GAIN / GRAMS FEED	WEIGHT' GAIN, GM.	FEED' UPTAKE, GM.	GRAMS' GAIN / GRAMS FEED	WEIGHT KIDNEY, GM. ABSOLUTE	WEIGHT KIDNEY, GM. PER 100 GM. RAT	WEIGHT ADRENAL, MG. ABSOLUTE	WEIGHT ADRENAL, MG. PER 100 GM. RAT	WEIGHT THYROID, MG. ABSOLUTE	WEIGHT THYROID, MG. PER 100 GM. RAT	FEMUR LENGTH, MM.
(2)	109.3	750	.156				1.7314	.648	73.1	27.4	21.4	8.1	29.0
14	179.9	750	.239										
(3)	114.9	705	.164	23.3	378	.061							
19	186.6	705	.264	−1.4	378	−.004							
(4)	109.1	560	.194				1.7343	.713	52.4	22.2	14.6	6.0	28.1
12	176.6	560	.315				2.7637	.887	49.2	15.7	19.8	6.3	30.2
(5)	72.2	654	.110	39.1	367	.106							
30	127.5	654	.195	30.9	367	.090							
(7)	77.0	744	.102				1.7045	.660	60.6	23.4	22.9	8.8	29.6
15	180.5	744	.242				2.6683	.741	48.5	17.9	24.8	6.6	31.4
(8)	89.1	744	.120				1.7418	.650	63.1	23.4	13.3	5.1	29.6
23	180.7	744	.243										
MEAN"	96.2	693	.141	31.2	372	.083	1.7280	.668	62.3	24.1	18.5	7.0	29.1
	166.4	693	.202	14.7	372	.043	2.7160	.814	48.8	16.8	22.3	6.4	30.8
STANDARD DEVIATION"	16.7	68	.033				.0100	.026	7.4	2.0	4.2	1.5	.7
	20.7	68	.028				.0480	.073	.3	1.1	2.5	.2	.6
STANDARD ERROR" (MEAN)	6.9	28	.013				.0050	.013	3.7	1.0	2.1	.7	.3
	8.4	28	.011				.0340	.052	.2	.8	1.2	.1	.4

PAIRS
○ INDICATES EXPERIMENTAL ANIMAL
(2) - 14
(3) - 19
(4) - 12
(5) - 30
(7) - 15
(8) - 23
' NORMAL DIET
" EXPERIMENTAL VALUES UNDERLINED

GROUP IA

UNILATERAL NEPHRECTOMY + 4% DIETARY NH_4Cl
FIRST PERIOD 32 DAYS. SECOND PERIOD 15 DAYS.

RAT NUMBER	WEIGHT GAIN, GM.	FEED UPTAKE, GM.	GRAMS GAIN / GRAMS FEED	WEIGHT GAIN, GM.	FEED UPTAKE, GM.	GRAMS GAIN / GRAMS FEED	WEIGHT KIDNEY, GM. ABSOLUTE	WEIGHT KIDNEY, GM. PER 100 GM. RAT	WEIGHT ADRENAL, MG. ABSOLUTE	WEIGHT ADRENAL, MG. PER 100 GM. RAT	WEIGHT THYROID, MG. ABSOLUTE	WEIGHT THYROID, MG. PER 100 GM. RAT	FEMUR LENGTH, MM.
(9)	180.2	809	.225	46.0	473	.097							
13	156.5	809	.195	− 3.1	473	−.007							
(17)	128.2	594	.216				1.6793	.546	68.3	22.3	30.5	9.9	29.8
18	147.5	594	.249				2.4260	.720	61.4	18.1	12.8	3.8	30.7
(21)	159.1	684	.233	37.7	460	.082							
20	196.0	684	.286	24.4	460	.053							
(22)	156.4	759	.206	29.7	464	.064							
29	133.8	759	.176	29.4	464	.063							
(25)	122.3	725	.169				1.5953	.454	63.5	18.2	25.7	7.4	31.2
24	142.9	725	.197										
(26)	117.0	517	.226				1.6229	.611	57.1	21.4	29.5	11.1	28.8
28	133.8	517	.257										
(27)	120.7	754	.163				1.9387	.628	62.5	20.2	26.3	8.6	30.2
16	144.1	754	.191				2.9695	.846	87.9	24.5	32.5	9.2	30.4
MEAN"	140.6	690	.205	37.8	466	.081	1.7090	.560	62.8	20.5	28.0	9.2	30.0
	150.1	690	.222	16.9	466	.036	2.6977	.783	74.6	21.4	22.6	6.5	30.5
STANDARD DEVIATION"	22.6	95	.026				.1360	.077	3.6	1.5	2.1	1.2	.8
	20.6	95	.035				.1360	.063	13.2	3.3	9.8	2.7	.1
STANDARD ERROR" (MEAN)	8.5	33	.010				.0680	.038	1.8	.8	1.0	.6	.4
	7.8	33	.013				.0960	.044	9.4	2.3	6.9	1.2	.1

PAIRS
○ INDICATES EXPERIMENTAL ANIMAL
(9) - 13
(17) - 18
(21) - 20
(22) - 29
(25) - 24
(26) - 28
(27) - 16
" EXPERIMENTAL VALUES UNDERLINED

GROUP IB

UNILATERAL NEPHRECTOMY
FIRST PERIOD 32 DAYS. SECOND PERIOD 18 DAYS.

Figure 7. Results of analyses of electrolytes of serum, muscle, and bone.

SUMMARY OF DATA

RAT NUMBER	SERUM pH	SERUM ELECTROLYTES Na, MEQ/L	K, MEQ/L	CO_2, MEQ/L	Cl, MEQ/L	P, MG.%	MUSCLE ELECTROLYTES FAT, GM.%	Na °	K °	P °	Cl °	BONE ELECTROLYTES Ca ×	Na ×	K ×	Cl ×
(2)	7.53	141.2	3.8	25.6	95.6	7.7	4.66	7.22	43.2	36.6	4.88	7834	173	44.4	15.8
(4)	7.31	144.6	3.7	17.7	110.0	9.2	5.74	7.99	48.8	40.0	6.08	6911	117	43.0	20.0
12	7.44	143.4	4.1	27.0	95.0	9.0	5.19	8.52	46.5	37.5	5.48	7481	114	42.6	23.9
(7)	7.30	140.6	4.1	18.1	109.0	8.2	5.19	8.02	45.3	36.7	5.39	7121	111	44.9	14.3
15	7.47	138.9	5.4	22.9	98.0	7.6	5.00	7.62	44.0	35.0	5.26	7562	152	51.4	21.6
(8)	7.55	141.2	4.7	28.0	98.0	8.3	4.89	7.83	46.4	35.8	5.10	6970	95	43.8	7.1
MEAN /	7.42	141.9	4.0	22.7	103.1	8.3	5.12	7.76	45.9	37.3	5.36	7204	126	44.2	14.3
	7.46	141.1	4.7	24.9	96.5	8.3	5.09	8.07	45.2	36.2	5.37	7521	133	47.0	22.7
STANDARD DEVIATION /	.12	1.4	.5	4.5	6.4	.5	.40	.32	2.0	1.6	.45	369	29	.7	4.7
	.02	2.2	.2	2	1.5	.7	.09	.45	1.2	1.2	.11	40	19	4.4	1.2
STANDARD ERROR (MEAN) /	.06	.7	.2	2.2	3.2	.2	.20	.16	1.0	.8	.22	184	14	.3	2.3
	.01	1.6	.1	1.4	1.1	.5	.06	.32	.8	.8	.08	28	13	3.1	.8

PAIRS
○ INDICATES EXPERIMENTAL ANIMAL
(2) - 14
(3) - 19
(4) - 12
(5) - 30
(7) - 15
(8) - 23

/ EXPERIMENTAL VALUES UNDERLINED

GROUP I A

UNILATERAL NEPHRECTOMY + 4% DIETARY NH_4Cl
° MMOL / 100 GM. FAT FREE SOLID
× MEQ / 1000 GM. FRESH BONE

RAT NUMBER	SERUM pH	SERUM ELECTROLYTES Na, MEQ/L	K, MEQ/L	CO_2, MEQ/L	Cl, MEQ/L	P, MG.%	MUSCLE ELECTROLYTES FAT, GM.%	Na °	K °	P °	Cl °	BONE ELECTROLYTES Ca ×	Na ×	K ×	Cl ×
(17)	7.31	142.5	3.8	23.9	97.6	8.9	7.61	9.23	49.5	40.0	6.84	7500	162	63.9	21.8
18	7.35	142.5	4.1	26.7	94.2	10.0	3.63	8.57	51.4	40.3	5.56	7876	165	36.4	19.7
(25)	7.41	143.1	4.1	24.8	99.0	7.9	7.60	9.68	49.3	39.2	6.28	6970	58	17.0	17.3
(26)	7.39	142.5	4.1	25.9	99.0	8.7	4.91	8.66	49.3	38.0	5.83	8030	200	39.2	14.4
(27)	7.21	144.4	5.4	34.6	92.0	9.6	9.24	7.53	45.3	35.2	4.86	7444	81	16.8	24.5
16	7.31	144.4	4.1	32.1	97.6	8.7	4.06	8.50	47.1	36.5	5.58	7380	156	50.5	11.3
MEAN /	7.33	143.1	4.3	27.3	96.9	8.8	7.34	8.77	48.3	38.1	5.95	7761	125	34.2	19.5
	7.33	143.4	4.1	29.4	95.9	9.3	3.85	8.53	49.2	38.4	5.57	7628	160	43.4	15.5
STANDARD DEVIATION /	.84	.8	.6	4.2	1.7	.6	1.60	.80	1.8	1.8	.79	447	184	19.4	3.9
	.02	1.0	0	2.7	1.7	.6	.21	.03	2.1	3.6	.01	248	4	7.1	4.2
STANDARD ERROR (MEAN) /	.42	.4	.3	2.1	.8	.3	.80	.40	.9	.9	.39	223	92	9.7	1.9
	01	.7	0	1.9	1.2	.4	.15	.02	1.5	2.5	.01	176	3	5.0	3.0

PAIRS
○ INDICATES EXPERIMENTAL ANIMAL
(9) - 13
(17) - 18
(21) - 20
(22) - 29
(25) - 24
(26) - 28
(27) - 16

/ EXPERIMENTAL VALUES UNDERLINED

GROUP I B

UNILATERAL NEPHRECTOMY

° MMOL / 100 GM. FAT FREE SOLID
× MEQ / 1000 GM. FRESH BONE

GROUP IA

	Rat No.	Na mEq/Kg Fresh Bone	K mEq/Kg Fresh Bone
Experimental Animals	2	152	37.9
	4	93	36.0
	7	94	39.4
	8	86	41.0
Control Animals	12	82	33.1
	15	124	41.4
Mean *		106	38.6
		103	37.2
Standard Deviation *		27	1.6
		21	4.1
Standard Error* (Mean)		13	.8
		15	2.9

GROUP IB

Experimental Animals	17	133	51.1
	25	35	14.4
	26	181	34.1
	27	47	12.9
Control Animals	16	141	45.3
	18	138	29.6
Mean *		99	28.1
		139	37.4
Standard Deviation*		60	15.7
		2	7.9
Standard Error * (Mean)		30	7.8
		1	5.5

Figure 7a. Bone sodium and potassium corrected for Donnan effect and for amounts of these electrolytes in vascular fluids.
*Acidotic values underlined.

Group II 4% NH_4Cl and 0.2% Diamox
Data for this group appear in Figures 8 to 12 inclusive

Growth

The experimental animals in this group gained an average of 3.1 grams daily for a total average increment of 125.8 grams over the forty-day initial experimental period. During that time, they consumed an average of 21.1 grams of feed daily for a total average feed uptake of 843 grams for the forty-day period. Their nutritional efficiency averaged 0.154.

During the same period of time, the control animals, although restricted to the same dietary intake as the experimental animals, gained an average of 4.8 grams daily for a total average weight increment of 191.1 grams. Their nutritional efficiency averaged 0.231. Thus, the control animals on the same amount of feed gained half again as much weight as the experimental animals.

An error was made during the second part of this experiment. Instead of giving only the experimental animals the altered diet, which contained an added 13.1 per cent of various electrolytes, all animals received this diet. This produced some unexpected results.

During this period of fourteen days, the experimental animals gained an average of approximately 1 gram daily for a total average increment of 13.7 grams. These animals consumed 29.5 grams of feed daily during this period for a total feed uptake of 416 grams. Their nutritional efficiency averaged 0.045. The controls, meanwhile, receiving the same amount of special diet, lost an average of 3.1 grams daily for a total average loss of 43.2 grams.

The growth curves will serve to demonstrate the pattern of growth. It will be seen that both groups of animals gained weight at the same rate until the experimental animals were started on 1 per cent ammonium chloride. At that time, these animals experienced a sharp loss of weight, but quickly made up for lost time. Four days later, the amount of ammonium chloride was doubled and 0.5 per cent Diamox was added to the diet. The animals then experienced a gradual slowing of the rate of growth, maximum within seven days of modification of the diet, but they soon recovered from the effects of the added acid-producing substances in the diet.

Therefore, sixteen days after the start of the experiment, the level of dietary acid-producing substances was doubled. This caused a marked diminution of the rate of growth of the experimental animals from which they did not recover.

At the end of the initial period, a diet calculated to be electrolytically neutral was started for both experimental and control animals. This change of diet had little effect on the experimental animals, which, after a slight loss of weight, kept on gaining at the same rate as before. The control animals, however, lost weight continually on this diet.

Organs

The kidneys of the experimental animals averaged 2.4435 grams in weight. The relative weight of kidney tissue (per 100 grams body weight) in these animals averaged 0.920 grams. The kidneys of the control animals weighed an average of 2.6619 grams, or 0.752 grams per 100 grams of body weight. Thus, the kidneys of the experimental animals averaged 22 per cent heavier per unit of body weight than those of the controls.

The adrenals of the experimental animals weighed an average of 51.0 mg, or 19.3 mg per 100 grams body weight.

The adrenals of the controls, however, weighed an average of 45.3 mg, or 12.7 mg, per 100 gram rat. The adrenals of the experimental animals, therefore, weighed 52 per cent heavier than those of the controls per unit of body weight.

The thyroid glands of the experimental animals weighed an average of 23.0 mg, or 8.6 mg, per 100 grams body weight. The thyroids of the controls, although they weighed an average of 32.5 mg, weighed only 8.9 mg per 100 grams of rat. Thus, there was no significant difference in the weight of thyroid per unit of body weight between experimental and control animals.

The length of the femurs of the experimental animals averaged 29.1 mm. Those of the control animals averaged 31.6 mm in length. The experimental animals, therefore, had femurs 9 per cent shorter than their normal controls.

Histology

Sections of thyroid glands of both experimental and control animals showed no abnormalities of structure. Follicles were lined with low columnar epithelium and contained normal colloid.

Two sections of thyroid glands of experimental animals revealed normal parathyroid tissue.

Adrenal glands, on section, showed normal histological structure in both groups of animals.

There were no differences noted on examination of sections of kidneys between experimental and control animals. Size of organ, secretory tubules, and glomeruli were all normal.

Sections of the distal end of the femur showed abnormalities in every experimental animal. These changes were only moderate in degree and consisted of osteoporosis in three animals and osteitis fibrosa in four animals.

Roentgen Examination

Radiological examination of one pair of animals revealed no marked abnormalities of the skeleton of the experimental animals, in spite of the histological abnormalities revealed by sections of bone. There was slight decrease of the radio-density of bone in these animals. No other changes could be discerned.

Electrolytes

Serum:

Serum analysis revealed the experimental animals in this group to have a moderate degree of hyperchloremic acidosis.

Serum pH of the experimental animals averaged 7.22, while that of the controls averaged 7.46. Carbon dioxide content of the serum averaged 17.2 mEq/L in the experimental animals, and 23.9 mEq/L in the controls. In addition, chloride concentration in the serum averaged 107.3 mEq/L in the experimental animals, and 98.0 mEq/L in the controls.

There was no significant difference between experimental and control animals in the serum concentrate of sodium, potassium, and inorganic phosphorus.

Muscle:

Analyses of muscle showed an average sodium concentration of 7.36 mM per 100 gram fat free solid in the acidotic animals and 7.05 mM per 100 gram fat free solid in the controls. Potassium content in acidotic animals averaged 42.6 mM per 100 gram fat free solid, and in the controls, 45.0 mM per 100 gram fat free solid. The inorganic phosphorus content of muscle in the acidotic animals was slightly lower (35.6 mM per 100 gram fat free solid) than that of the controls (37.0 mM per 100 gram fat free solid). Chloride content was elevated in acidotic animals over controls. Thus, the controls averaged 5.20 mM chloride per 100 gram fat free solid, while the acidotic animals averaged 5.71 mM chloride per 100 gram fat free solid.

There was less fat in the muscle of acidotic animals than in the controls. Thus, controls averaged 5.36 gram of fat per 100 gram solid, and acidotic animals averaged 4.18 gram of fat per 100 gram solid.

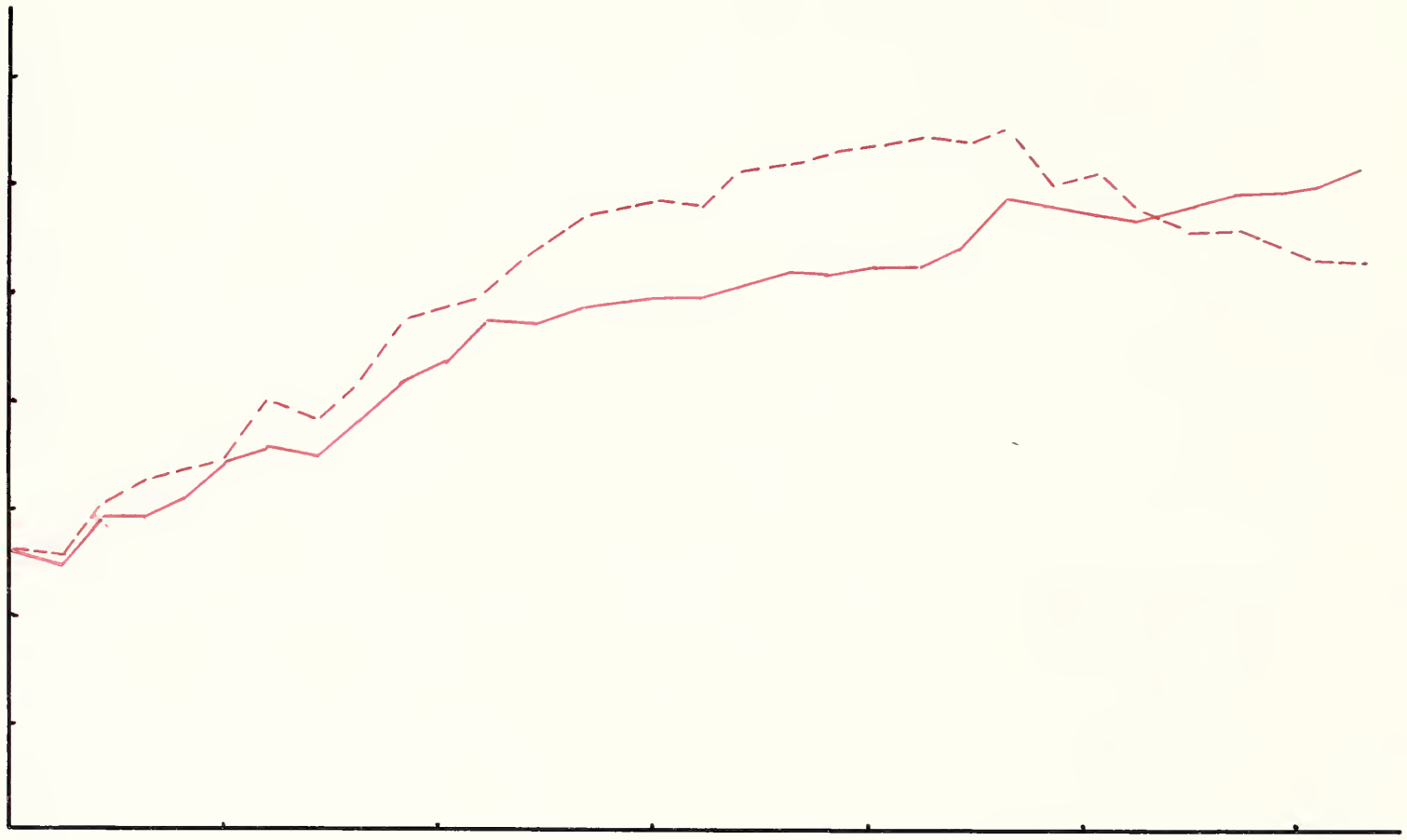
Bone:

Analyses of bone revealed that bone calcium was significantly lower in the acidotic animals (6644 mEq/Kg fresh bone) than in the controls (7507 mEq/Kg fresh bone). Bone sodium was unexpectedly higher in the acidotic animals [194 (corrected 159) mEq/Kg fresh bone] than it was in the controls [176 (corrected 140) mEq/Kg fresh bone]. There were no significant differences between experimental animals and controls with regard to average bone potassium [52.5 (corrected 39.9) mEq/Kg fresh bone vs. 37.2 (corrected 25.6) mEq/Kg fresh bone]. The same held true for bone chloride. Thus, bone chloride in acidotic animals averaged

28.1 mEq/Kg fresh bone, while bone chloride averaged 24.9 mEq/Kg fresh bone in the control animals.

The differences in average bone sodium, potassium, and chloride, while seeming large, are not significant (see Figure 22).

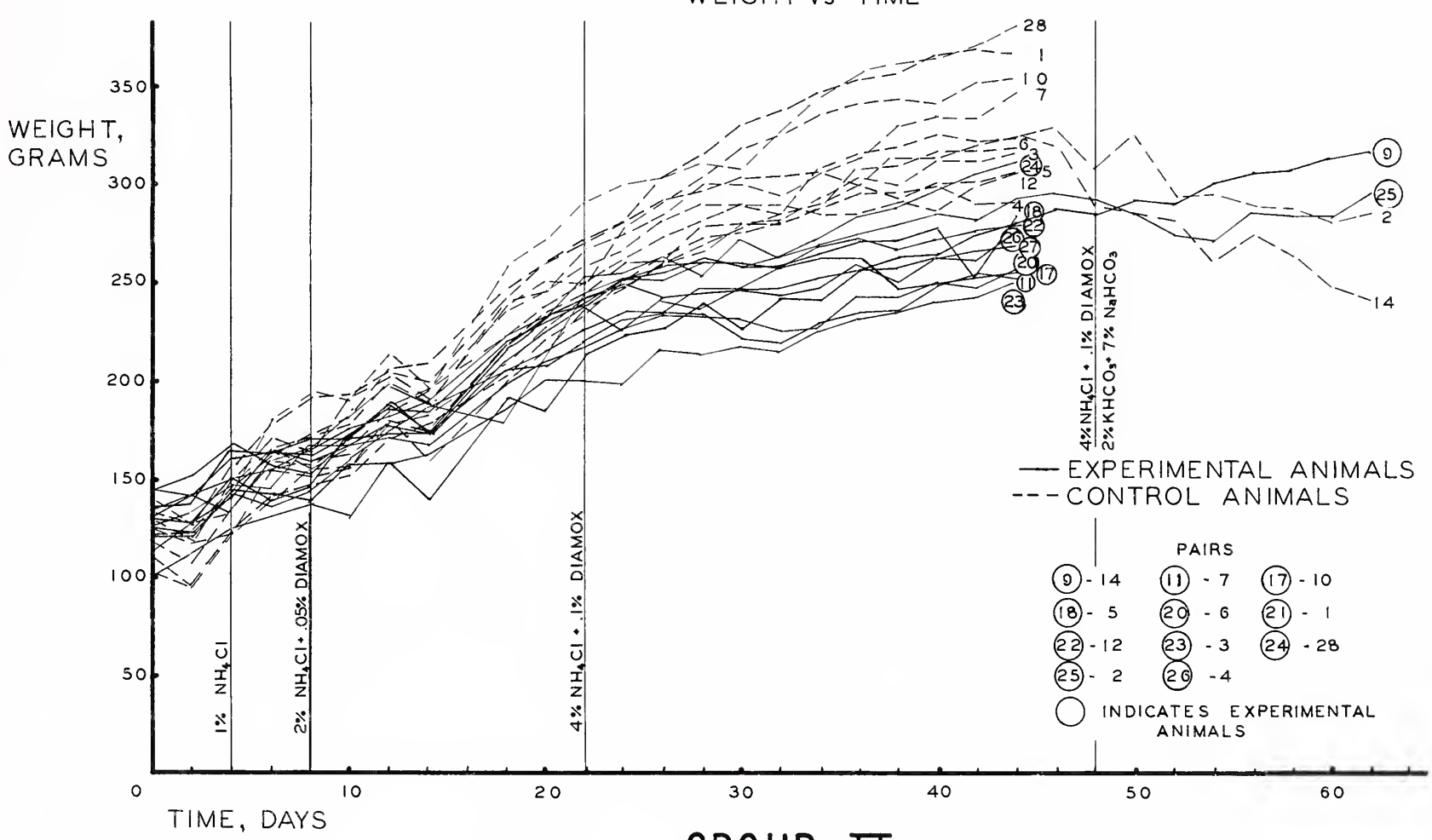
Figure 8. Individual growth curves for all animals. The red curves show median growth for experimental and control animals.



MEDIAN WEIGHT VS TIME

MEDIAN WEIGHT VS TIME

GROWTH DATA



GROUP II

4 % NH_4 Cl + .1% DIAMOX

(DIETARY)

Figure 9. Radiogram of animals 22 and 12. Experimental animal on left, control on right. No marked differences in radio-density are observed.

Figure 10. Growth, feed, and organ data.

SUMMARY OF DATA

RAT NUMBER	WEIGHT GAIN, GM.	FEED UPTAKE, GM.	GRAMS GAIN / GRAMS FEED	WEIGHT GAIN,' GM.	FEED UPTAKE,' GM.	GRAMS' GAIN / GRAMS FEED	WEIGHT KIDNEY, GM.		WEIGHT ADRENAL, MG.		WEIGHT THYROID, MG.		FEMUR LENGTH, MM.
							ABSOLUTE	PER 100 GM. RAT	ABSOLUTE	PER 100 GM. RAT	ABSOLUTE	PER 100 GM. RAT	
(9)	138.1	861	.160	38.1	465	.082							
14	201.1	861	.234	−64.4	465	−.113							
(11)	110.0	738	.149				2.3332	.928	46.4	18.4	14.7	5.8	27.9
7	196.2	738	267				2.7159	.787	43.4	12.6	28.5	8.2	31.4
(17)	95.3	853	.111				2.2115	.873	58.1	22.9	27.3	10.6	29.6
10	204.9	853	.239				2.4633	.700	38.7	10.9	38.7	10.5	31.7
(18)	115.0	893	.128										
5	147.2	893	.165										
(20)	110.7	796	.141				2.5152	.986	59.0	23.2	22.1	8.6	30.2
6	182.6	796	.231										
(21)	116.6	900	.129				2.3614	.889	32.9	12.8	24.8	9.3	28.4
1	220.4	900	.248				2.8066	.768	53.7	14.6	30.3	8.2	31.7
(22)	113.1	883	.128	11.9	392	.031							
12	172.2	883	.195	−43.2	392	−.110							
(23)	125.0	808	.154				2.4781	.998	49.8	20.1	25.4	10.1	28.2
3	197.6	808	.243										
(24)	182.0	905	.202				2.6854	.859	60.3	19.4	23.5	7.5	30.2
28	238.8	905	.264										
(25)	145.5	760	.191	9.0	391	.023							
2	180.7	760	.246	−22.0	391	−.056							
(26)	136.1	875	.156				2.5199	.910	50.7	18.2	23.3	8.4	29.3
4	169.7	875	.194										
MEAN"	125.8	843	.154	13.7	416	.045	2.4435	.920	51.0	19.3	23.0	8.6	29.1
	191.1	843	.231	−43.2	416	−.093	2.6619	.752	45.3	12.7	32.5	8.9	31.6
STANDARD DEVIATION"	22.5	55	.030				.1430	.033	8.9	3.2	3.4	1.5	.9
	24.2	55	.030				.1450	.037	5.5	1.5	4.2	1.1	.1
STANDARD ERROR (MEAN)"	6.8	17	.009				.0550	.013	3.3	1.2	1.3	.6	.3
	7.3	17	.009				.0850	.021	3.1	.8	2.4	.6	.1

PAIRS

○ INDICATES EXPERIMENTAL ANIMAL

(9) - 14
(11) - 7
(17) - 10
(18) - 5
(20) - 6
(21) - 1
(22) - 12
(23) - 3
(24) - 28
(25) - 2
(26) - 4

'NEUTRAL DIET

"EXPERIMENTAL VALUES UNDERLINED

GROUP II

4% NH_4Cl + .1% DIAMOX

FIRST PERIOD 40 DAYS. SECOND PERIOD 14 DAYS

Figure 11. Results of analyses of serum, muscle, and bone electrolytes.

SUMMARY OF DATA

RAT NUMBER	SERUM	SERUM ELECTROLYTES					MUSCLE ELECTROLYTES					BONE ELECTROLYTES			
	pH	Na, MEQ/L	K, MEQ/L	CO_2, MEQ/L	Cl, MEQ/L	P, MG. %	FAT, GM. %	Na °	K °	P °	Cl °	Ca ×	Na ×	K ×	Cl ×
(11)		144.6	4.5	18.0	106.8	9.4	3.94	6.48	43.2	35.2	4.89	7639	235	38.9	12.9
7	7.50	142.4	3.9	23.5	100.0	8.4	5.41	7.15	44.7	36.0	5.19	7497	198	35.3	30.3
(17)	7.17	144.6	3.9	20.1	104.7	9.7	2.89	7.33	43.2	36.8	5.79	6100	149	51.6	27.9
10	7.40	141.1	4.5	24.4	96.0	9.0	5.16	7.46	44.9	36.5	5.16	7333	198	38.8	18.8
(20)	7.20	143.4	4.1	15.5	108.0	9.5	3.95	7.10	43.5	34.8	5.30	6245	175	62.6	30.1
(21)	7.37	144.5	4.4	18.9	101.8	9.0	5.77	6.99	42.7	37.3	5.20	6821	181	37.3	27.6
1	7.49	143.4	4.5	23.9	98.0	8.1	5.51	6.54	45.4	37.6	5.25	7690	132	37.4	25.6
(23)	7.18	145.7	4.1	15.4	112.0	9.4	5.61	8.40	42.0	35.2	6.52	6242	162	51.9	35.7
(24)		142.3	4.2	17.5	106.0	9.2	3.25	7.82	43.7	34.9	6.55	7174	201	52.0	28.3
(26)	7.30	142.3	4.5	13.1	112.0		3.87	7.39	40.1	34.9	5.72	6284	252	73.0	34.3
MEAN /	7.22	143.9	4.2	17.2	107.3	9.5	4.18	7.36	42.6	35.6	5.71	6644	194	52.5	28.1
	7.46	142.3	4.3	23.9	98.0	8.5	5.36	7.05	45.0	37.0	5.20	7507	176	37.2	24.9
STANDARD DEVIATION /	.08	1.2	.2	2.2	3.6	.3	1.00	.57	1.2	.9	.58	541	35	11.5	6.9
	.04	.9	.2	.3	1.6	.3	.15	.38	.3	.2	.17	216	31	1.4	4.7
STANDARD ERROR (MEAN) /	.03	.4	.1	.8	1.4	.1	.38	.22	.5	.3	.22	206	13	4.3	2.6
	.02	.5	.1	.2	.9	.2	.09	.22	.2	.1	.10	124	18	.8	2.7

PAIRS

○ INDICATES EXPERIMENTAL ANIMAL

(9) - 14
(11) - 7
(17) - 10
(18) - 5
(20) - 6
(21) - 1
(23) - 12
(23) - 3
(24) - 28
(25) - 2
(26) - 4

/ EXPERIMENTAL VALUES UNDERLINED

GROUP II

4 % NH_4Cl + .1% DIAMOX

° MMOL/100 GM. FAT FREE SOLID
× MEQ/1000 GM. FRESH BONE

GROUP II

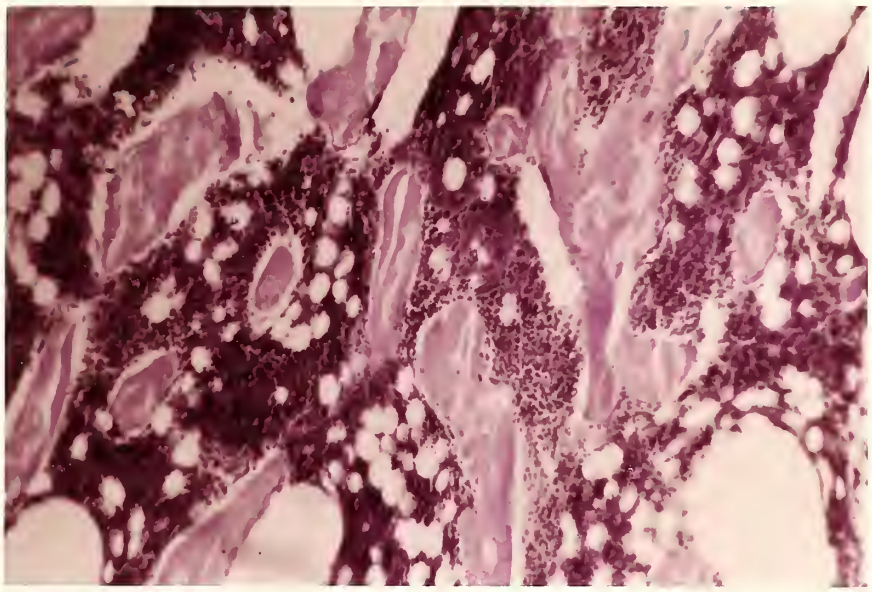
	Rat No.	Na mEq/Kg Fresh Bone	K mEq/Kg Fresh Bone
Experimental Animals	11	218	34.7
	17	114	39.4
	20	139	47.1
	21	146	28.2
	23	120	37.2
	24	167	39.6
	26	213	53.0
Control Animals	1	98	29.7
	7	159	25.7
	10	163	32.0
Mean*		<u>159</u> 140	<u>39.9</u> 25.6
Standard Deviation *		<u>39</u> 30	<u>6.8</u> 4.4
Standard Error* (Mean)		<u>15</u> 17	<u>2.6</u> 2.5

<u>Figure 11a.</u> Bone sodium and potassium corrected for Donnan effect and for amounts of these electrolytes in vascular fluids.
* Acidotic values underlined.

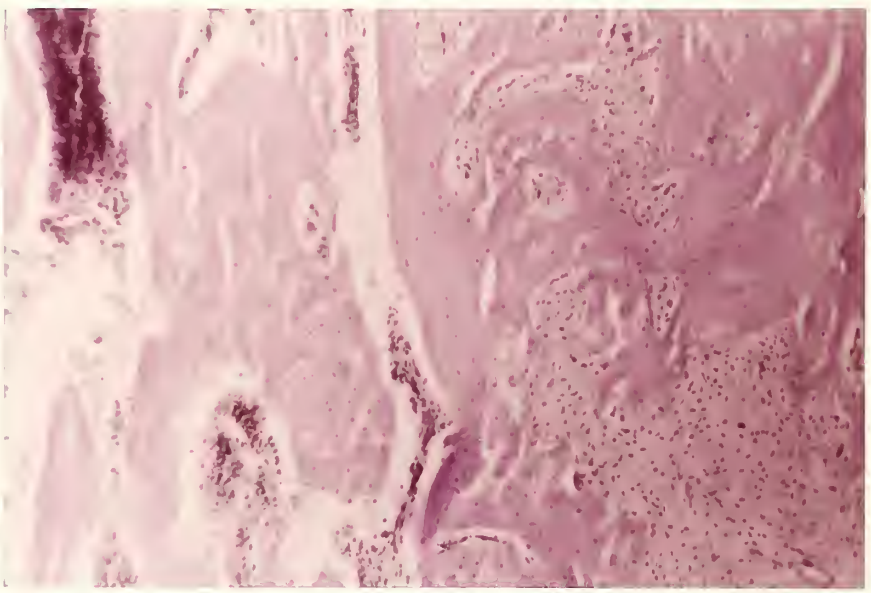
Figure 12. Longitudinal section of distal end of femur. Hematoxylin and Eosin.

A. Control Animal - normal marrow.

B. Experimental Animal - note diffuse fibrosis of marrow cavity.



A



B

Group III 13% Carbon Dioxide
Data for this group appear in Figures 13 to 16, inclusive

Growth

The experimental animals in Group III gained an average of approximately 1 gram daily during the initial experimental period of thirty-eight days for a total weight increment of 39.1 grams. During this time, they consumed an average of 12.7 grams of feed daily for a total average feed uptake of 482 grams. Their nutritional efficiency averaged 0.080.

The control animals, although limited to the same feed consumption, gained an average of 4.3 grams daily for a total average weight increment of 120.8 grams. Their nutritional efficiency was therefore three times as great as the experimental animals, i.e. 0.252.

During the second normal period of sixteen days, the experimental animals gained an average of 6.7 grams daily for a total average increment of 106.6 grams. This weight gain was made on an average daily feed uptake of 23.7 grams. Total feed consumption for this period averaged 382 grams. Nutritional efficiency of these animals averaged 0.279. The control animals, during the same period and on the same feed uptake, gained an average of 4.5 grams daily for a total average weight gain of 72.5 grams. The nutritional efficiency of these animals averaged 0.172.

The growth curves reveal that the experimental animals were growing slightly faster than the controls at the start of the acid-producing regimen. As soon as 13 per cent carbon dioxide was instituted, however, the slope of the growth curves leveled off for both groups of animals,

the experimental animals to a greater degree than the controls. These animals (controls) soon recovered, and in six days were growing at approximately the normal rate. The experimental animals, however, grew very little thereafter in the atmosphere of carbon dioxide.

As soon as the carbon dioxide content in the air was diminished to normal, both groups underwent a period of enhanced growth, most marked in the experimental animals. At the termination of this period, the weight of the experimental animals almost equalled that of the controls.

Organs

The kidneys of the experimental animals averaged 2.0150 grams in weight, or 1.090 grams per 100 grams of body weight. In the control animals, however, the kidneys averaged 2.0198 grams in weight, or 0.769 grams per 100 grams of rat. Thus, the experimental animals had 42 per cent more renal tissue per unit of body weight than their normal controls.

The adrenal glands of the experimental animals weighed an average of 53.4 mg, or 28.9 mg per 100 grams of rat. The adrenals of the controls, however, weighed an averaged of 54.1 mg, or 20.6 mg per 100 grams body weight of rat.

The thyroid glands of the experimental animals averaged 18.7 mg in weight, or 10.2 mg per 100 grams of rat. The thyroids of the controls weighed 26.2 mg, or 9.9 mg per 100 grams body weight, on the average. Thus, no significant difference in weight of thyroid glands was observed between experimental and control animals.

Histology

Sections of thyroid glands of both experimental and control animals revealed follicles lined with low columnar epithelium and filled with normal colloid.

Sections of thyroid glands in three animals revealed normal parathyroid tissue.

The adrenal glands, on histological examinations, revealed normal cellular architecture in both experimental and control animals.

Sections of kidneys of experimental animals revealed no abnormalities of glomeruli or tubules.

Bone sections of the distal end of the femur revealed skeletal changes ranging from osteoporosis in 85 per cent to osteitis fibrosa, mild in degree, in 15 per cent.

Roentgen Examination

Radiological examination of one pair of animals revealed changes characteristic of slight osteitis fibrosa and osteoporosis in the experimental animals. These changes may be described as decreased radio-density involving the appendicular skeleton, with slight distortion of the trabecular pattern best demonstrated at the distal end of the femur and proximal end of the tibia. No rachitic changes were noted, save for slight widening of the epiphysial plate, seen at the distal end of radius and ulna.

Electrolytes

Serum:

Serum analysis of the experimental animals in this group showed them to be suffering from a severe respiratory acidosis.

Serum pH of the experimental animals averaged 7.11 while that of the controls averaged 7.35.

The serum electrolytes showed changes typical of severe respiratory acidosis. Thus, the serum carbon dioxide content of the experimental animals averaged 50.0 mEq/L, while that of the controls averaged a normal 23.6 mEq/L. Serum chloride levels in the experimental animals were decreased to an average of 78.1 mEq/L, while that of the controls averaged 99.9 mEq/L. There was no significant difference in serum sodium, potassium and inorganic phosphorus concentrations between experimental and control groups.

Muscle:

No significant differences were observed between acidotic and control animals with regard to muscle sodium and potassium. Muscle chloride, however, was decreased in the acidotic animals. Thus, average chloride content in the controls was 6.09 mM per 100 gram fat free solid, while that of the acidotic animals averaged 4.73 mM per 100 gram fat free solid. The content of muscle inorganic phosphorus was also lower in the acidotic animals (37.1 mM per 100 gram fat free solid) than in the controls (39.4 mM per 100 gram fat free solid).

Fat was markedly decreased in acidotic animals, being approximately one half that of the controls. Thus, the average fat content of acidotic muscle was 1.84 gram fat per 100 gram solid, while that of the controls was 3.71 gram fat per 100 gram solid.

Bone:

Analyses of bone revealed average bone calcium to be somewhat lower

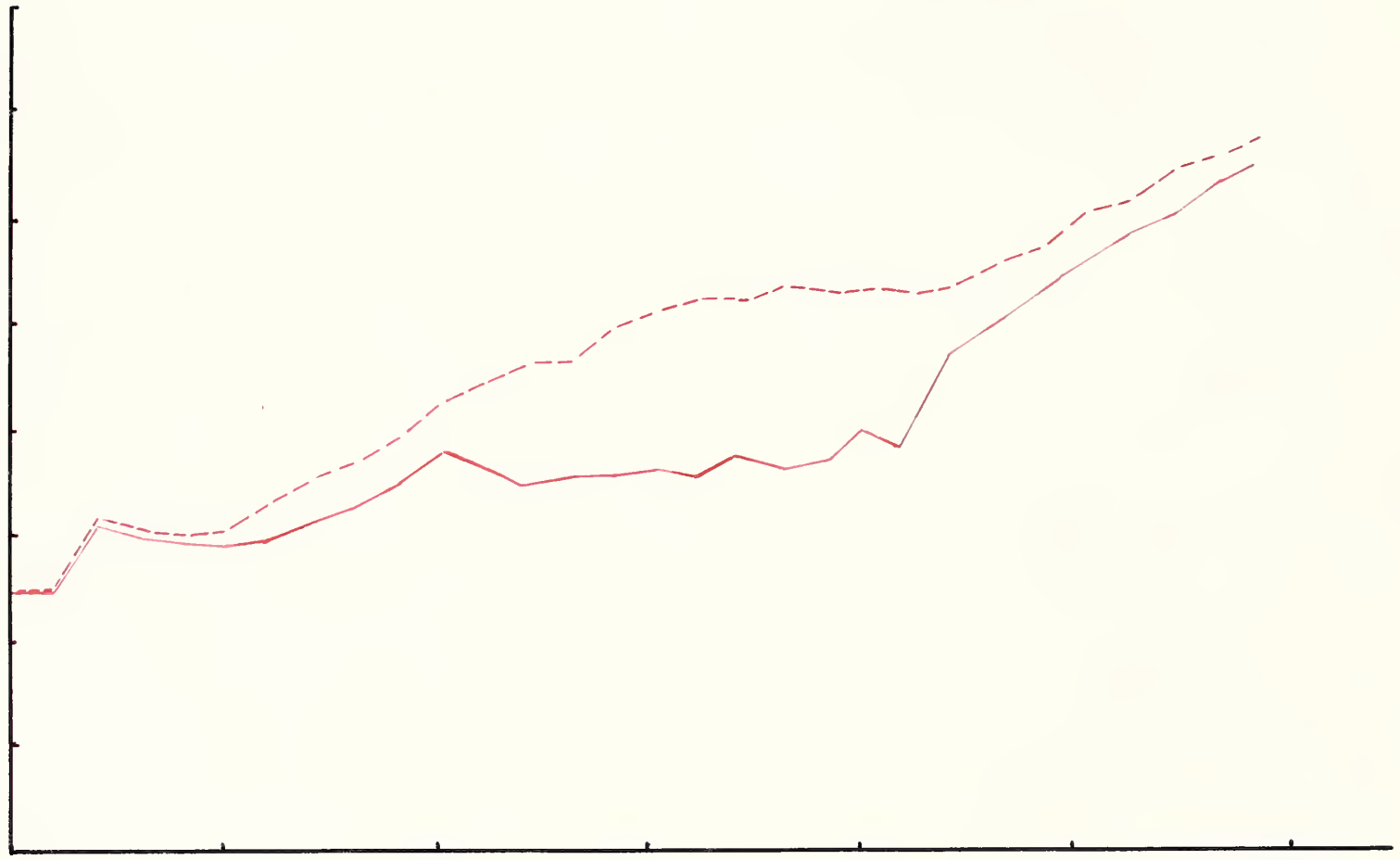
in the acidotic animals (7836 mEq/Kg fresh bone) than in the controls (7946 mEq/Kg fresh bone).

Bone sodium was also slightly lower in the acidotic animals. Thus, the acidotic animals averaged .149 (corrected 121) mEq sodium/Kg fresh bone, while the controls averaged 156 (corrected 126) mEq sodium/Kg fresh bone.

Bone potassium averaged 36.6 (corrected 29.6) mEq/Kg fresh bone in the acidotic animals, while the controls had an average of 61.8 (corrected 49.0) mEq of potassium/Kg fresh bone.

Average bone chloride was significantly lower in the acidotic animals (18.4 mEq/Kg fresh bone) than in the controls (22.5 mEq/Kg fresh bone).

Figure 13. Individual growth curves for all animals. The red curves show median growth for experimental and control animals.

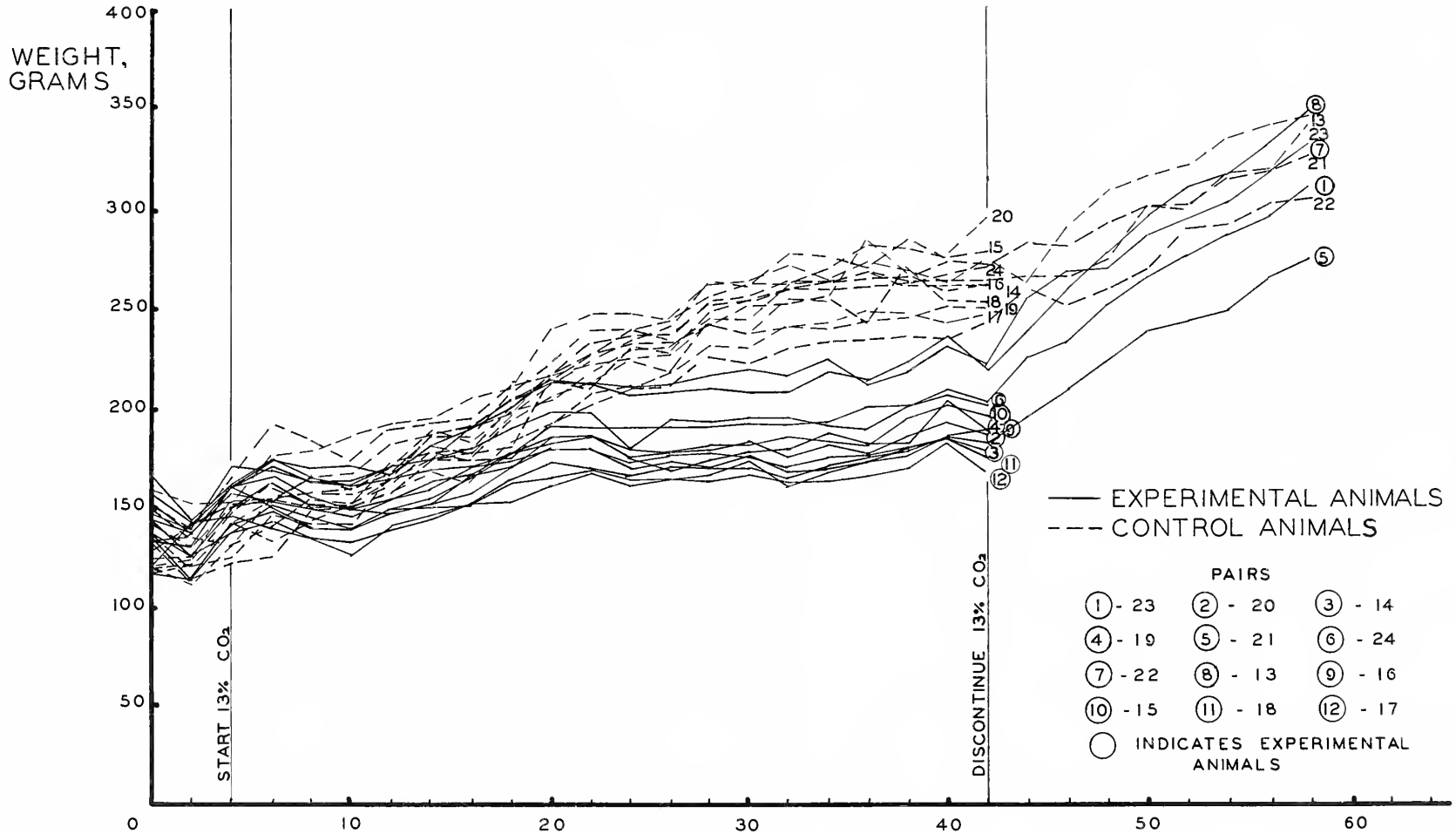


MEDIAN WEIGHT VS TIME

MEDIAN WEIGHT VS TIME

GROWTH DATA

WEIGHT vs TIME



GROUP III

13% CARBON DIOXIDE

Figure 14. Radiogram of animals 4 and 19. Experimental animal on right, control on left. Note decreased radio-density and small size of skeleton in experimental animal.

Figure 15. Growth, feed, and organ data.

SUMMARY OF DATA

RAT NUMBER	WEIGHT GAIN, GM.	FEED UPTAKE, GM.	GRAMS GAIN / GRAMS FEED	WEIGHT GAIN,′ GM.	FEED UPTAKE,′ GM.	GRAMS GAIN′ / GRAMS FEED	WEIGHT KIDNEY, GM.		WEIGHT ADRENAL, MG.		WEIGHT THYROID, MG.		FEMUR LENGTH, MM.
							ABSOLUTE	PER 100 GM. RAT	ABSOLUTE	PER 100 GM. RAT	ABSOLUTE	PER 100 GM. RAT	
(1)	46.6	490	.095	115.2	374	.310							
23	107.7	490	.219	70.3	374	.188							
(2)	48.1	512	.094				2.0608	1.120	49.0	26.4	22.1	11.9	27.2
20	142.5	512	.278										
(3)	28.7	441	.065				2.0710	1.160	49.2	27.6	18.0	10.1	27.6
14	130.0	441	.294				2.0451	.784	53.4	20.6	19.5	7.5	29.0
(4)	42.0	480	.088										
19	107.6	480	.224										
(5)	44.7	500	.089	78.4	366	.214							
21	113.3	500	.226	67.5	366	.184							
(6)	43.1	549	.079				2.0852	1.020	55.7	27.3	15.0	7.4	27.6
24	143.5	549	.262				1.9822	.743	51.4	19.1	26.2	9.7	29.6
(7)	52.1	473	.110	119.2	399	.300							
22	122.0	473	.258	69.6	399	.175							
(8)	54.2	507	.107	113.4	388	.292							
13	120.9	507	.241	82.5	388	.211							
(9)	48.1	447	.108				1.9392	1.010	51.1	27.1	17.7	9.4	26.8
16	130.0	447	.292				2.0322	.780	57.6	22.1	33.0	12.6	29.8
(10)	35.9	533	.067				2.0062	1.020	51.0	26.5	19.5	10.1	27.4
15	133.0	533	.249										
(11)	14.2	434	.032				1.9153	1.080	56.6	31.9	19.0	10.8	26.3
18	104.3	434	.242										
(12)	12.3	415	.030				2.0214	1.180	61.3	35.1	19.4	11.4	26.2
17	95.1	415	.229										
MEAN″	39.1	482	.080	106.6	382	.279	2.0150	1.090	53.4	28.9	18.7	10.2	27.0
	120.8	482	.252	72.5	382	.172	2.0198	.769	54.1	20.6	26.2	9.9	29.4
STANDARD DEVIATION″	13.0	39	.026				.0610	.066	4.2	3.1	1.9	1.3	.5
	15.0	39	.024				.0260	.017	2.6	1.2	5.8	2.1	.6
STANDARD ERROR (MEAN)″	3.7	11	.007				.0230	.025	1.6	1.2	.7	.5	.2
	4.3	11	.007				.0150	.010	1.5	.7	3.3	1.2	3

PAIRS

○ INDICATES EXPERIMENTAL ANIMAL

(1) - 23
(2) - 20
(3) - 14
(4) - 19
(5) - 21
(6) - 24
(7) - 22
(8) - 13
(9) - 16
(10) - 15
(11) - 18
(12) - 17

′NORMAL ATMOSPHERE

″EXPERIMENTAL VALUES UNDERLINED

GROUP III

13% CARBON DIOXIDE

FIRST PERIOD 38 DAYS. SECOND PERIOD 16 DAYS.

Figure 16. Results of analyses of serum, bone, and muscle electrolytes.

SUMMARY OF DATA

RAT NUMBER	SERUM	SERUM ELECTROLYTES					MUSCLE ELECTROLYTES					BONE ELECTROLYTES			
	pH	Na_s, MEQ/L	K_s, MEQ/L	CO_2, MEQ/L	Cl_s, MEQ/L	P_s, MG.%	FAT, GM.%	Na°	K°	P°	Cl°	$Ca^{\times}$	$Na^{\times}$	$K^{\times}$	$Cl^{\times}$
(2)	7.03	146.3	3.6	54.9	76.2	10.1	1.40	9.27	49.7	37.3	5.18	8166	124	33.4	17.5
(3)	7.08	146.3	3.5	42.4	81.0	7.9	1.54	9.10	49.8	37.3	4.67	7904	124	12.5	22.2
14	7.41	140.0	3.9	21.8	100.0	7.7	2.89	9.01	49.2	38.3	6.54	8585	192		21.0
(6)	7.08	146.3	2.9	50.1	77.4	7.4	1.54	9.10	49.8	36.6	4.67	7585	127		17.2
24	7.28	145.6	3.8	23.9	98.2	9.4	4.02	8.77	51.7	40.1	5.81	7635	162	58.6	24.4
(9)	7.17	142.5	4.3	52.3	75.6	7.5	1.45	9.22	53.9	37.2	4.94	7764	199	61.0	18.2
16	7.36	146.9	3.8	25.0	101.6	8.6	4.23	9.58	51.0	39.9	5.92	7618	114	65.1	22.1
(10)	7.27	145.6	4.8	54.2	78.4	6.2	2.42	8.84	50.8	37.2	4.57	7730	167		21.5
(11)	7.07	145.6	2.3	46.2	85.0	2.8	2.63	8.77	52.3	39.8	5.06	7738	170	66.9	14.0
(12)	7.09			49.9	73.4		1.87	9.00	45.6	34.1	4.04	7965	132	9.3	17.7
MEAN /	7.11	145.4	3.6	50.0	78.1	7.1	1.84	9.04	50.3	37.1	4.73	7836	149	36.6	18.4
	7.35	143.2	3.8	23.6	99.9	8.6	3.71	9.12	50.6	39.4	6.09	7946	156	61.8	22.5
STANDARD DEVIATION /	.08	1.3	.8	4.1	1.9	.9	.46	.17	2.2	1.5	.35	214	27	23.6	2.6
	.05	2.8	.05	1.3	1.1	.7	.59	.34	1.1	.8	.30	452	32	3.3	1.4
STANDARD ERROR (MEAN) /	.03	.5	.3	1.5	.7	.4	.17	.06	.8	.6	.13	81	10	10.5	1.0
	.03	1.6	.03	.7	.6	.4	.34	.20	.6	.5	.17	262	18	2.3	.8

PAIRS

○ INDICATES EXPERIMENTAL ANIMAL

(1) - 23
(2) - 20
(3) - 14
(4) - 19
(5) - 21
(6) - 24
(7) - 22
(8) - 13
(9) - 16
(10) - 15
(11) - 18
(12) - 17

/ EXPERIMENTAL VALUES UNDERLINED

GROUP III

13% CARBON DIOXIDE

° MMOL/100 GM. FAT FREE SOLID
× MEQ/1000 GM. FRESH BONE

GROUP III

	Rat No.	Na mEq/Kg Fresh Bone	K mEq/Kg Fresh Bone
Experimental Animals	2	94	26.6
	3	88	8.9
	6	98	
	9	168	47.9
	10	131	
	11	149	57.1
	12		7.3
Control Animals	14	165	
	16	85	52.4
	24	129	45.6
Mean*		<u>121</u> 126	<u>29.6</u> 49.0
Standard Deviation*		<u>30</u> 33	<u>20.1</u> 3.4
Standard Error* (Mean)		<u>12</u> 19	<u>9.0</u> 2.4

<u>Figure 16a.</u> Bone sodium and potassium corrected for Donnan effect and for amounts of these electrolytes in vascular fluids.
*Acidotic values underlined.

Group IV 0.4% Diamox
Data for this group appear in Figures 17 to 19 inclusive

Growth

During the forty-two day period, the experimental animals gained an average of 4.4 grams per day for a total average weight gain of 184.8 grams. At the same time, they consumed an average of about 16 grams of feed daily for a total average feed consumption of 664 grams. Their nutritional efficiency averaged 0.279.

The control animals, during this period, gained an average of about 4.4 grams daily for a total average weight gain of 185.9 grams. They likewise consumed 15.8 grams feed daily, or 664 grams total. Thus, there was a difference of only 1.1 gram in total weight gain between the two groups of animals.

The growth curves of the two groups of animals paralleled each other closely. The experimental animals suffered a slight setback in weight gain on starting 0.2 per cent Diamox in their feed, but this was overcome in three days. For the next twenty-seven days, their median weight was slightly greater than that of the controls. This was in spite of the fact that the amount of Diamox they received was doubled on the seventeenth day, causing them a slight depression in weight gain.

Organs

The absolute weight of the kidney of the experimental animals averaged 2.4520 grams, while the kidneys of the controls averaged 2.3555 grams. The weight per 100 gram rat of kidney tissue averaged 0.903 gram in the experimental animals and 0.965 grams in the controls.

Thus, there was no striking difference in either absolute or relative weight of kidney between experimental and control animals.

The thyroids of the experimental animals averaged 24.6 mg, or 9.0 mg per 100 gram rat; those of the controls averaged 24.6 mg, or 9.5 mg per 100 gram rat. Here, likewise, the weight of thyroid is almost identical in the two groups.

No significant difference was observed between the average length of femur of the experimental animals (28.0 mm) and of the controls (28.8 mm).

Histology and Roentgen Examination

No radiological or histological studies were done on these animals.

Electrolytes

Serum:

Serum analyses showed the experimental animals to be suffering from a moderate degree of hyperchloremic acidosis. pH averaged 7.13 in the experimental animals and 7.36 in the controls. Carbon dioxide content averaged 19.8 mEq/L in the experimental animals and 24.7 mEq/L in the controls.

Chloride averaged 108.4 mEq/L in the experimental animals and 99.8 mEq/L in the controls. No significant difference was noted in the concentration of serum sodium and potassium between the two groups of animals.

The most interesting finding in the serum was the marked increase in the inorganic phosphate in the experimental animals. The inorganic

phosphate in the experimental animals averaged 11.1 mg per cent; that of the controls averaged 9.1 mg per cent. The difference is statistically significant.

Muscle:

No analyses of muscle electrolytes were done in this group.

Bone:

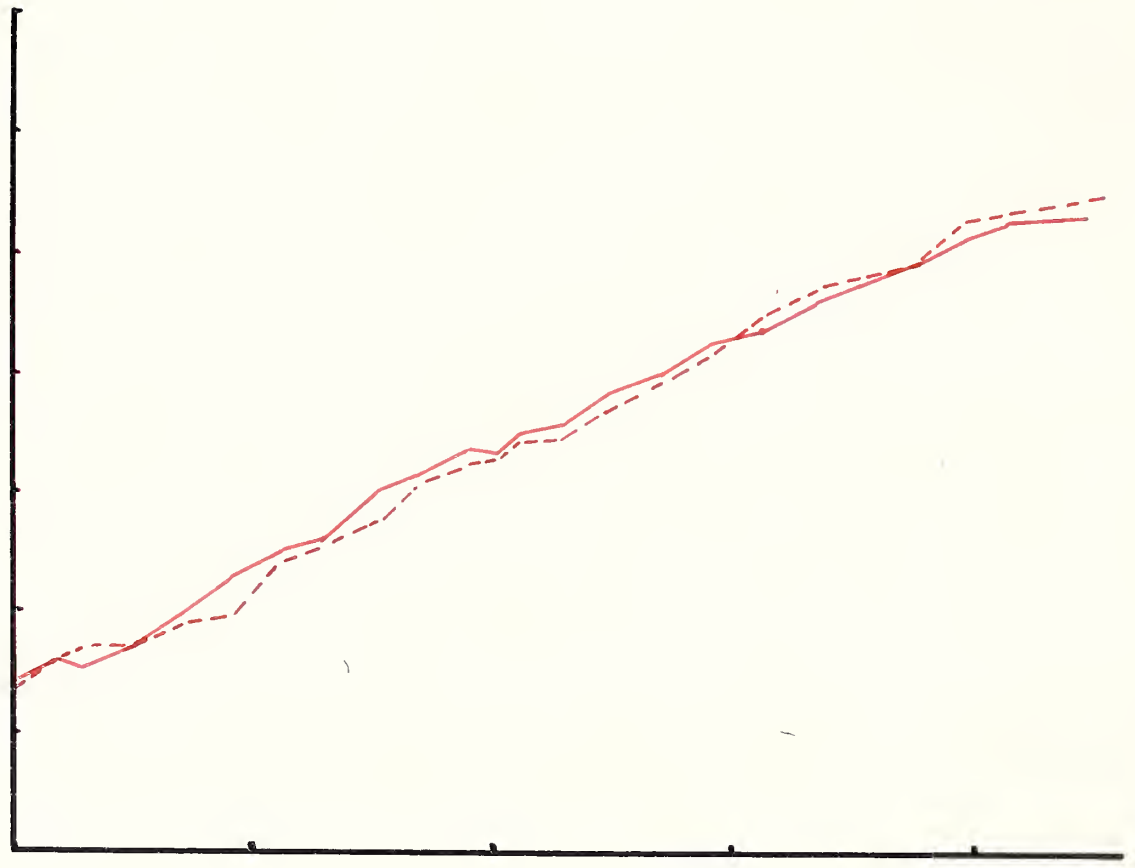
No analyses of bone electrolytes were done on the control animals in this group. Consequently, control values were taken from Group II, in which the control animals underwent normal growth. (In Group II, the controls were somewhat retarded in growth due to decreased food intake.)

Analyses of bone electrolytes showed the average bone calcium to be significantly lower in the acidotic animals (6592 mEq/Kg fresh bone) than in the controls (7507 mEq/Kg fresh bone).

Bone sodium was unexpectedly higher in the acidotic animals than in the controls. Thus, bone sodium averaged 196 (corrected 163) mEq/Kg fresh bone in the acidotic animals, as against 176 (corrected 140) mEq/Kg fresh bone in the controls. The same held true for bone potassium. In the acidotic animals, bone potassium averaged 88.3 (corrected 71.0) mEq/Kg fresh bone, while the controls had an average of 37.2 (corrected 25.6) mEq of potassium/Kg fresh bone.

Bone chloride in the acidotic animals averaged 27.1 mEq/Kg fresh bone, while that of the controls averaged 24.9 mEq/Kg fresh bone.

Figure 17. Individual growth curves for all animals. The red curves show median growth for experimental and control animals.

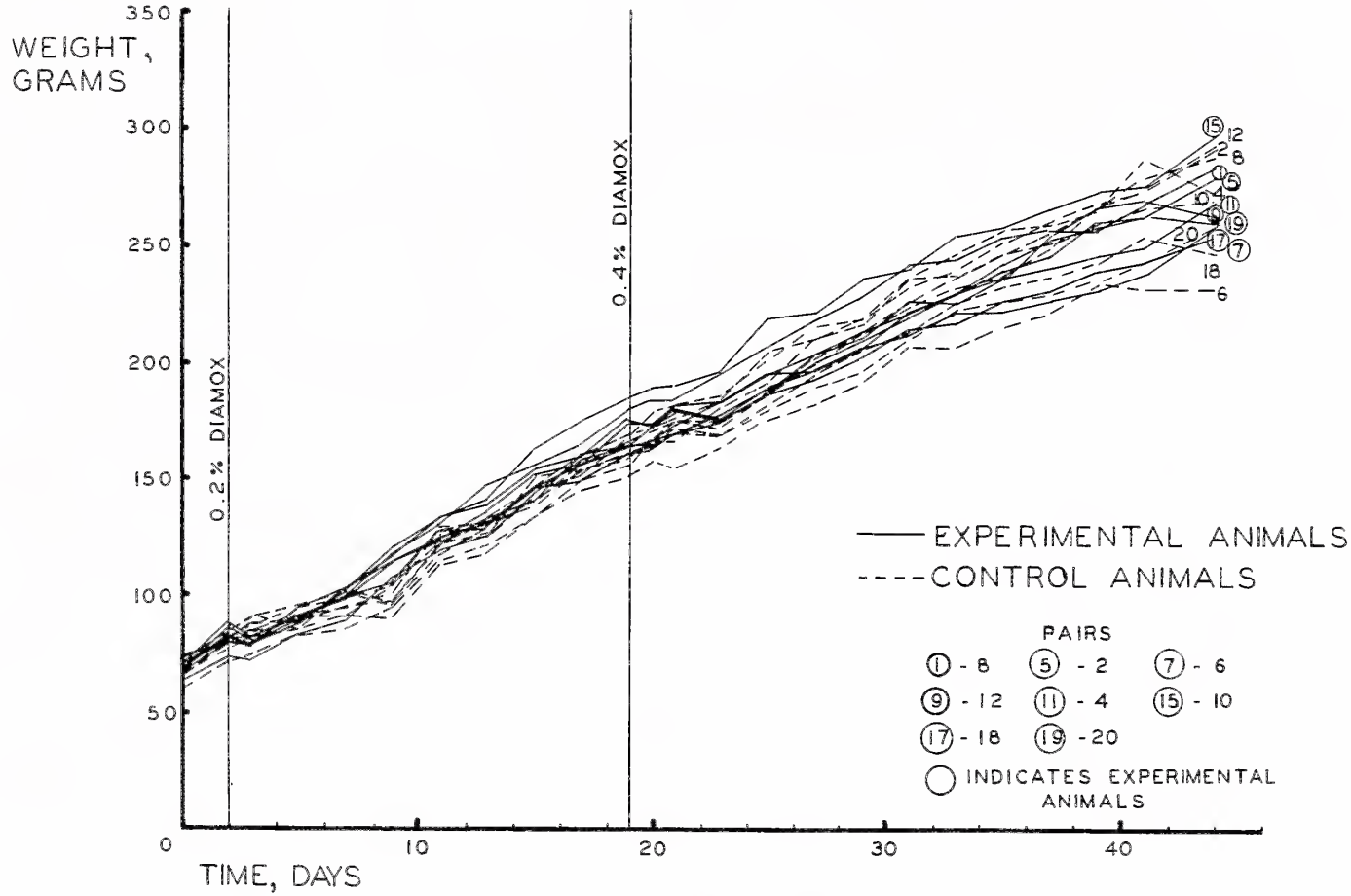


MEDIAN WEIGHT VS TIME

MEDIAN WEIGHT AS TIME

GROWTH DATA

WEIGHT vs TIME



GROUP IV

0.4 % DIAMOX

(DIETARY)

Figure 13. Growth, feed, and organ data.

SUMMARY OF DATA

RAT NUMBER	WEIGHT GAIN GM.	FEED UPTAKE GM.	GRAMS GAIN / GRAMS FEED	WEIGHT KIDNEY, GM. ABSOLUTE	WEIGHT KIDNEY, GM. PER 100 GM. RAT	WEIGHT ADRENAL, MG. ABSOLUTE	WEIGHT ADRENAL, MG. PER 100 GM. RAT	WEIGHT THYROID, MG. ABSOLUTE	WEIGHT THYROID, MG. PER 100 GM. RAT	FEMUR LENGTH, MM.
(1)	201.0	650	.309							
8	202.1	650	.310							
(5)	188.5	671	.281							
2	206.5	671	.306							
(7)	168.3	604	.280							
6	151.3	604	.250							
(9)	186.5	679	.276							
12	217.0	679	.320							
(11)	184.5	661	.280	2.3250	.878	60.7	22.9	19.3	7.3	28.2
4	190.6	661	.288	2.2533	.838	59.8	22.2	15.5	5.7	29.5
(15)	205.0	694	.296	2.6977	.928	62.2	21.6	27.8	9.6	30.0
10	183.6	694	.265	2.2053	.778	55.0	20.7	25.9	9.7	29.5
(17)	171.5	629	.278	2.2667	.828	49.6	19.6	26.1	10.2	28.2
18	162.5	629	.266	2.6094	1.320	49.4	20.2	27.6	11.2	28.2
(19)	173.0	724	.238	2.5188	.979	48.2	18.8	22.8	8.9	29.5
20	174.0	724	.240	2.3542	.924	47.9	18.7	29.3	11.4	28.2
MEAN /	184.8	664	.279	2.4250	.903	55.2	20.7	24.0	9.0	28.9
	185.9	664	.281	2.3555	.965	53.0	20.4	24.6	9.5	28.8
STANDARD DEVIATION /	12.8	35	.019	.170	.060	6.3	1.6	3.2	1.1	.1
	20.8	35	.026	.160	.210	4.7	1.3	5.8	2.3	.1
STANDARD ERROR / (MEAN)	4.5	12	.007	.085	.030	3.1	.8	1.6	.6	.04
	7.4	12	.009	.080	.100	2.3	.6	2.9	1.1	.04

PAIRS
◯ INDICATES EXPERIMENTAL ANIMAL

(1) - 8
(5) - 2
(7) - 6
(9) - 12
(11) - 4
(15) - 10
(17) - 18
(19) - 20

/ EXPERIMENTAL VALUES UNDERLINED

GROUP IV

0.4% DIAMOX (DIETARY)

EXPERIMENTAL PERIOD 42 DAYS.

Figure 19. Results of analyses of serum, bone, and muscle electrolytes.

SUMMARY OF DATA

RAT NUMBER	SERUM pH	SERUM ELECTROLYTES Na, MEQ/L	K, MEQ/L	CO_2, MEQ/L	Cl, MEQ/L	P, MG. %	MUSCLE ELECTROLYTES FAT, GM. %	Na °	K °	P °	Cl °	BONE ELECTROLYTES Ca *	Na *	K *	Cl *
(1)	7.10	146	3.1	17.6	106.4	11.2									
8	7.32	139	5.3	25.1	102.8	8.6									
(5)	7.10	143	4.8	19.6	109.6	11.0									
2	7.38	143	4.8	24.9	99.6	8.7									
(7)	7.08	144	4.6	18.9	109.6	10.3									
6	7.31	142	4.6	22.6	104.0	9.1									
(9)	7.09	143	4.0	18.8	110.4	11.2									
12	7.35	143	4.0	22.2	99.6	9.9	NO ANALYSES OF MUSCLE WERE DONE IN GROUP IV								
(11)	7.11	146	4.0	22.0	107.6	11.4						6132	226	58.0	34.8
4	7.36	142	4.1	28.1	98.4	9.2									
(15)	7.19	142	4.3	18.7	109.2	10.7						6659	190	89.0	28.2
10	7.37	141	4.8	26.1	99.2	9.3									
(17)	7.20	146	3.3	20.9	108.9	10.4						6595			29.9
18	7.36	145	3.7	23.9	99.6	10.1									
(19)	7.22	143	3.7	20.8	106.4	11.3						6981	173	118.0	15.4
20	7.44	143	3.7	24.3	100.0	9.1									
MEAN'	7.13	144	4.0	19.8	108.4	11.1						6592	196	88.3	27.1
	7.36	142	4.4	24.7	99.8	9.1						7507	176	37.2	24.9
STANDARD DEVIATION'	.05	1.5	.6	1.5	1.4	.4						303	22	24.5	6.9
	.01	1.6	.5	1.8	1.9	.5						216	31	1.4	4.7
STANDARD ERROR (MEAN)'	.01	.5	.2	.5	.5	.1						151	13	14.2	3.4
	.00	.6	.2	.6	.7	.2						124	18	.8	2.7

PAIRS

◯ INDICATES EXPERIMENTAL ANIMAL

(1) - 8
(5) - 2
(7) - 6
(9) - 12
(11) - 4
(15) - 10
(17) - 18
(19) - 20

' EXPERIMENTAL VALUES UNDERLINED

GROUP IV

0.4 % DIAMOX (DIETARY)

° MMOL / 100 GM. FAT FREE SOLID
* ME Q / 1000 GM. FRESH BONE

CONTROL VALUES FOR BONE ELECTROLYTES TAKEN FROM GROUP II

GROUP IV

	Rat No.	Na mEq/Kg Fresh Bone	K mEq/Kg Fresh Bone
	11	179	41.9
Experimental	15	157	38.5
Animals	17		
	19	154	102.7
Mean*		<u>163</u> 140	<u>71.0</u> 25.6
Standard Deviation *		<u>11</u> 30	<u>24.9</u> 4.4
Standard Error* (Mean)		<u>6</u> 17	<u>14.4</u> 2.5

Figure 19a. Bone sodium and potassium corrected for Donnan effect and for amounts of these electrolytes in vascular fluids.
*Acidotic values underlined.

Fecal Nitrogen and Fat Data appear in Figure 20.

In these animals made acidotic by 4 per cent ammonium chloride and 0.1 per cent Diamox, no significant differences were noted between experimental and control animals with regard to amount of feces produced per unit weight of feed, amount of fecal nitrogen, or amount of fecal fat.

Hourly Variations in pH and Electrolytes

Data appear in Figure 21.

No consistant trends are seen in analyses of serum electrolytes at different hours of the day. There seems to be a gradual decrease in pH as the day progresses, which is probably too small to be significant. However, it does indicate that the rats do not become less acidotic as the night approaches.

Statistical Significance of all Data

The standard error of the difference between the experimental and control means compared to the difference between experimental and control means appears in Figure 22.

	Rat No.	Weight Feces (dried) / Weight Feed Consumed	Fecal Nitrogen (Per 100g. Dried Feces)	Fecal Fat (Per 100g. Dried Feces)
Control Animals	1	0.222	4.98	3.53
	2.	0.212	5.08	3.05
	3.	0.241	5.42	2.74
Experimental Animals	4.	0.226	4.78	4.76
	5.	0.248	5.20	3.50
	6.	0.212	4.71	2.35
Mean*		0.229	4.89	3.54
		0.225	5.16	3.11
Standard Deviation*		0.011	0.22	0.71
		0.010	0.19	0.32
Standard Error*		0.004	0.07	0.23
(Mean)		0.003	0.06	0.11

Figure 20. Fecal nitrogen and fat of acidotic animals compared to controls.

*Acidotic values underlined.

Time Killed	Rat No.	pH	Na	K	Cl	CO_2
10:00 a.m.	3	7.15	140.7	3.3	114.4	19.2
	4	7.20	140.7	3.2	112.2	19.9
	Average	7.18	140.7	3.2	113.3	19.6
2:00 p.m.	1	7.19	141.4	3.9	111.6	19.2
	2	7.12	141.4	3.9	106.0	23.2
	Average	7.16	141.4	3.5	108.8	21.2
5:00 p.m.	5	7.12	138.5	3.9	108.4	22.7
	6	7.12	138.5	3.9	108.4	18.1
	Average	7.12	138.5	3.9	108.4	20.9
11:00 p.m.	7	Died before exsanguination completed				
	8	7.27	142.9	3.5	114.0	18.7

Figure 21. Changes in pH and serum electrolytes at approximately four-hour intervals during the day. Two animals killed at times specified. Animals made acidotic with 0.4 per cent Diamox in feed.

DISCUSSION

Growth

In the first three groups of animals, acidosis was produced by the introduction into the internal environment of exogenous substance, i.e., ammonium chloride or carbonic acid. In Group IV, acidosis was produced by interfering with the body's mechanism of conserving fixed cation. It is important to keep these two experimental mechanisms in mind, in view of the different results obtained. In both of these groups, a chronic acidosis, existing during the major part of the animals' growth period, was produced.

In Groups IA, II, and III, retarded growth, roughly proportional to the degree of acidosis, was produced in the experimental animals. This was characterized not only by less weight gain, but also by smaller skeletons. In addition to this, the bones were poorly calcified in those animals which suffered the most marked acidosis, especially in Groups II and III, as shown by radiological and histological evidence.

Other workers have experimented with the effect of acid producing substances on growth and metabolism.

Meneely (1952) observed that diets containing 9.8 per cent sodium chloride caused decreased weight gains over a twenty-week period in rats. He advanced no explanation for this phenomenon.

Addis et al (1926) observed that 2 per cent calcium chloride in diet (which acts as an acid-producing substance because the calcium is not absorbed from gastro-intestinal tract) produced retarded growth as

measured by both weight gain and skeletal length in rats. He also found decreased urinary protein and increased urinary ammonia nitrogen. An incidental finding was that heart weight was considerably smaller in acidotic animals than in controls. This finding was unexpected at the start of the experiment and unexplained at the end.

Jaffe et al (1932) showed that ammonium chloride ingestion with low calcium diet produced retarded growth in dogs.

Burns (1929) showed that 0.1 ammonium chloride in the diet of rats caused decreased growth.

None of the above workers utilized a paired feeding technique in their experiments, so that nutritional factors may complicate the picture. However, it seems that acid-producing substances, such as dilute hydrochloric acid, ammonium chloride, and calcium chloride have some effect on growth, and a more marked effect on metabolism of bone, causing rickets or osteitis fibrosa.

In the present experiments, nutritional factors were kept identical (within experimental error) so that any effect on growth was due to acidosis per se.

In these animals, the metabolic response to acidosis produced renal hypertrophy. Certain workers have shown that, if rats are dwarfed by various means, various organs such as kidney, thyroid, or adrenal, do not share the retarded growth of the body as a whole, and therefore, the weight of e.g. kidney tissue per 100 gram of rat is greater than that of the normal controls (Smith, 1931). Whether an organ such as the kidney is hypertrophied or not depends on the experimental procedure. Thus, decreased mineral intake, decreased water intake or increased

protein intake (with unilateral nephrectomy) caused renal hypertrophy. That in the present experiments renal hypertrophy occurred is indicated by the fact that the remaining kidneys of the acidotic animals in Group IA were both absolutely and relatively larger than those of IB, in which no acidosis occurred, indicating greater hypertrophy. In addition, relative weights of thyroid tissue were similar in both experimental and control animals in all three groups. Adrenal tissue in the acidotic animals of Groups IA, II, and III was also relatively greater than in the controls.

In Group IV, however, where acidosis was produced by interfering with renal mechanisms for acidifying urine and thus conserving fixed cation (by inhibiting carbonic anhydrase), no retarded growth was produced even though the acidosis was just as severe as in Group II. An interesting finding which may throw some light on this matter is the fact that serum inorganic phosphorus was 20 per cent higher in the experimental animals of the group. No such consistent elevation of phosphorus was observed in the experimental animals of the other groups. Talbot (1952) has observed that in humans with anterior pituitary dysfunction characterized by increased circulating pituitary growth hormone (PGH) i.e. acromegaly, serum inorganic phosphorus is raised and this fact may be used as a test for increased amounts of circulatory PGH.

Li (1949) observed that serum inorganic phosphorus decreased with age in rats, and also that hypophysectomy caused a decrease in serum inorganic phosphorus. This decrease could be corrected by administering PGH.

In Group IV, it is possible that something in the experimental procedure stimulated the anterior pituitary to produce increased amounts of PGH, thus overcoming any tendency to dwarfism. The fact that there was no splanchromegaly (as is seen in human acromegaly) may be explained by the fact that the stimulus of increased PGH occurred during the normal growing period of the animals and thus an overall increased growth occurred. The most interesting recent work which bears on this aspect of the problem is that of Fazekas (1953), who fed virgin female rabbits acid-ash substances for three weeks, with a rest period of one week. He found that profuse lactation occurred after eight to nine weeks of this treatment. He postulated that the slight shift of the acid-base equilibrium to the acid side stimulated the anterior pituitary, causing lactation. Further studies on this aspect of the problem are indicated.

Electrolytes

Studies show that in Groups IA, II, and IV, in spite of hyperchloremic acidosis with decreased carbon dioxide levels, serum sodium and potassium concentrations were normal in both experimental and control animals. Moreoever, in Group III, serum sodium and potassium were also normal in the experimental animals in spite of severe respiratory acidosis. This occurred in spite of what must have been severe renal losses of these ions in the acidotic animals. However, this is not too surprising. Bergstrom and Wallace (1954) found that large amounts of sodium were present in the mineral structure of bone and about one-third of the sodium seemed to be interchangeable with that of the extracellular fluid. This skeletal sodium amounts to 30 per cent of total body sodium in the rat.

Many workers have investigated thoroughly the effects of acidosis on metabolism of bone.

Jaffe and Bodanski (1932) showed that ammonium chloride acidosis with decreased dietary calcium resulted in rickets in juvenile dogs and osteitis fibrosa in adult dogs. If calcium was present in normal levels in the diet, bone calcification was more nearly normal in spite of ingestion of ammonium chloride.

Farquharson et al (1939) showed that acid increased the excretion of calcium in urine, but they considered this to be a minor factor in the neutralization of excess acid, which apparently is corered by an increase in urinary ammonia nitrogen. In addition, ketogenic diets caused increased urinary losses of calcium. In these cases, fecal calcium remained constant despite low calcium intake (100 mg calcium per day). This would indicate that the calcium lost in metabolic acidosis comes from mobilization of body calcium and not from an increase in absorption of calcium. No studies have been done on calcium balance in respiratory acidosis.

Goto (1918) found, in rabbits fed 0.25N HCl, that the most marked skeletal change was decreased fat, and that calcium carbonate was slightly decreased.

In the present experiments, bone calcium was decreased in all the animals suffering hypoerchloremic acidosis, i.e. the experimental animals in Groups IA, II, and IV. There was no significant alteration of bone calcium in respiratory acidosis. In view of the increased losses of calcium in the urine, it is interesting that there were no evidences of calcification in the renal parenchyma observed during histological examination of the kidneys.

The chlorides of bone seemed to follow the serum chlorides. Thus, high bone chlorides were found in those animals suffering hyperchloremic acidosis, and low bone chlorides were found in those animals with respiratory acidosis.

There was considerable variation from the mean in the analyses of bone sodium and potassium. Consequently, no conclusion may be drawn from the results of these determinations. In fact, values just the opposite of the expected ones were found in Groups II, III, and IV, where the acidotic animals had higher bone sodium content than the controls. One factor adding to the confusion in these analyses is the fact that the reagents, e.g. ammonium chloride (C.P.) and nitric acid (C.P.) were found to be contaminated with sodium. This was also found by Bergstrom and Wallace (1954) who found it necessary to purify their reagents.

Muscle fat was low in Groups II and III, in which there was the most marked acidosis and the most marked retardation of growth. This decrease in body fat was obvious grossly, on post-mortem examination of the animals. The significance of this change in acidosis is unknown. Hormonal factors are known to influence deposition of body fat, however. Thus, Li (1949) demonstrated in rats that excess PGH causes decreased body fat; moreover, excess corticotropin (ACTH) causes increased deposition of body fat.

Cooke et al (1952) observed that rats suffering from chronic respiratory acidosis had low muscle sodium and content of muscle potassium at the upper limits of normal. In addition, muscle chloride was low in the experimental animals suffering respiratory acidosis.

Muscle chloride was significantly lower in the experimental animals in Group II in the present experiments. No significant changes were seen in muscle sodium or potassium.

In those animals suffering hyperchloremic acidosis in Group II, the muscle chlorides were higher and muscle potassium and inorganic phosphorus were lower than normal.

Differences in caloric balance have been considered as a reason for the different weight gains in experimental and control animals. However, in this experiment, no such differences were observed. Thus, feed uptake of experimental and control animals was equal (within the limits of experimental error).

In addition, activity was similar in experimental and control animals in all groups save III, where it was the acidotic animals which exhibited decreased activity. Moreover, in a small group of animals made acidotic by ammonium chloride and Diamox, feces collected for a three-day period were analyzed for nitrogen and fat. No significant differences were observed either in the fecal nitrogen or fat between acidotic and control animals.

The only avenue for loss of nitrogen which was not explored was urinary. Addis et al (1926) found that 2 per cent calcium chloride in diet, which acts as e.g. hydrochloric acid because the calcium is not absorbed, caused increased urinary loss of nitrogen, especially in the ammonia nitrogen fraction. Conversely, urinary protein nitrogen in these animals was about half that of normal controls. In addition, both normal controls and animals fed sodium bicarbonate had many more red blood cells

in the urine than the acidotic controls. This is interesting in view of the work of Chambers and Zweifach (1940) on the effect of calcium and pH on the integrity of the capillary inter-cellular cement substance.

SUMMARY

Young male albino rats (Sprague Dawley) were subjected to the following procedures:

IA. Unilateral nephrectomy and 4 per cent ammonium chloride in diet.

IB. Unilateral nephrectomy.

II. 4 per cent ammonium chloride and 0.2 per cent Diamox diet.

III. 13 per cent carbon dioxide.

IV. 0.4 per cent Diamox in diet.

Pair fed controls were maintained under normal conditions. These various regimens produced acidosis which was hyperchloremic in Groups IA, II, and IV, and hypochloremic in Group III. In Groups IA, II, and III, retardation of growth, both in weight gain and femur length, was produced. This retardation in growth was roughly proportional to the degree of acidosis.

Additional changes produced by acidosis include: renal and adrenal hypertrophy (Groups IA, II, and III), decreased muscle fat (Groups II and III), and decreased bone calcium, most marked in those animals suffering from severe hyperchloremic acidosis (Groups IA, II, and IV).

However, in Group IV, in spite of acidosis, no dwarfism was produced during the experimental period of forty days.

The dwarfism was reversible under normal conditions in Groups IA, II, and III.

The results of this experiment seem to indicate that acidosis may have something to do with retardation of growth. However, more problems are created than solved by this work. Studies of hormone regulation of

growth; more accurate studies of intake and loss of nitrogen; and calorimetric studies of acidotic rats all remain to be done in order to elucidate the basic mechanism of the retardation of growth produced in these experiments.

STATISTICAL SIGNIFICANCE OF DATA

Group	IA	IB	II	III	IV
Weight Gain	-70.2 / 10.9	-9.5 / 11.6	-65.3 / 9.9	-81.7 / 5.7	-1.1 / 8.6
Nutritional Efficiency	-.061 / .017	-.017 / .016	-.077 / .013	-.172 / .001	-.002 / .011
Kidney Weight Absolute	-.9890 / .034	-.9837 / .117	-.2178 / .1017	-.0048 / .0274	+.0695 / .361
Kidney Weight Relative	-.146 / .054	-.223 / .058	+.168 / .025	+.321 / .027	-.062 / .104
Adrenal Weight Absolute	+13.5 / 3.7	-11.8 / 9.6	+5.7 / 4.5	-.7 / 2.4	+2.2 / 3.9
Adrenal Weight Relative	+7.3 / 1.3	-.9 / 1.3	+6.6 / 1.4	+8.3 / 1.4	+.3 / 1.0
Thyroid Weight Absolute	-3.8 / 1.6	+5.4 / 7.0	-10.5 / 2.7	-7.5 / 3.4	-.6 / 3.3
Thyroid Weight Relative	+.6 / .7	+2.7 / 1.3	-.3 / .8	+.3 / 1.3	-.5 / 1.2
Femur Length	-1.7 / .5	-.5 / .4	-2.5 / .3	-2.4 / .4	+.1 / .06
Serum pH	-.04 / .06	-.0 / .42	-.24 / .04	-.24 / .04	-.23 / .01
Serum Sodium	+.8 / 1.7	-.3 / .3	+1.6 / .6	+2.2 / 1.7	+2.0 / .8
Serum Potassium	-.7 / .2	+.2 / .3	-.1 / .1	-.2 / .3	-.4 / .5
Serum Carbon Dioxide	-2.2 / 2.6	-2.1 / 2.8	-6.7 / .8	+26.4 / 1.7	-4.9 / .3
Serum Chloride	+6.6 / 3.4	+1.0 / 1.4	+9.3 / 1.7	-21.8 / .9	+8.6 / .8

Figure 22. In the above fractions, the numerator is the difference between experimental and control means. The denominator is the standard error of this difference. If this quotient is greater than three, the difference between the means is considered statistically significant. (+ indicates experimental mean greater than control mean: - indicates experimental mean less than control mean.)

Group	IA	IB	II	III	IV
Serum Inorganic Phosphorus	.0 .5	-.5 .5	+1.0 .2	-1.5 .6	+2.0 .2
Muscle Fat	+.03 .66	+3.49 .81	-1.18 .39	-1.87 .38	
Muscle Sodium	-.31 .36	+.24 .40	+.31 .31	-.08 .20	
Muscle Potassium	+.7 1.3	-.9 1.7	-2.4 .5	-.3 1.0	
Muscle Inorganic Phosphorus	+1.1 1.1	-.3 2.6	-1.4 .3	-2.3 .8	
Muscle Chloride	-.01 .23	+.38 .39	+.51 .24	-.136 .21	
Bone Calcium	-317 186	+133 284	-863 240	-110 276	-315 196
Bone Sodium	-7 19	-35 92	+18 22	-7 21	+20 22
Corrected Bone Sodium	+3 20	-40 30	+19 23	-5 23	+23 18
Bone Potassium	-2.8 3.1	-9.2 10.9	+15.3 4.4	-25.2 10.8	+51.1 14.2
Corrected Bone Potassium	+1.4 3.0	-9.3 9.5	+14.3 3.6	-19.4 9.3	+45.4 14.6
Bone Chloride	-8.4 2.4	+4.0 3.6	+5.2 3.6	-4.1 1.3	+2.2 4.3
Weight Feces (Dried) / Weight Feed			+.004 .005		
Fecal Nitrogen			-.27 .09		
Fecal Fat			+.43 .25		

Figure 22. Continued.

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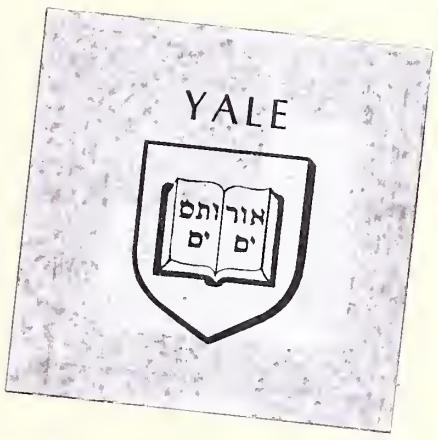
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